Synthesis methods for uniform particles of nano and micron sized apatite of various structures

By

Thomas S. Lawson

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Except where acknowledged in the customary manner, the material presented in this thesis is, to the best of my knowledge, original and has not been submitted in whole or part for a degree in any university.

Thomas S. Lawson

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Abstract

Inherently brittle materials can be somewhat be toughened by changing the way they are constructed. However, the nanostructures seen in materials such as nacre and the toughness that derives from them have yet to be repeated in the laboratory with artificial materials. One obstacle to their construction was the limited availability of bulk amounts of regular anisometric carbonate or apatite particles.

Therefore, the hypothesis of this work was to ask if it was feasible to make regular morphology nanoparticles of apatite in large amounts rapidly and at a low cost. This hypothesis was tested by undertaking three investigations. Investigations 1 and 2 utilised and extended the existing methods for making apatite, but did so in novel ways by combining the hydrothermal approach for producing regular particles with the precipitation approach for producing a high yield of particles. Investigation 3 then applied the insights gained from 1 and 2 to a method that used a domestic microwave to shorten the reaction time.

Investigation 1 sought to test if the particles could be made which were regular and unidirectional in shape. This work found that adding citric acid to the reaction gel and then ramping the incubation temperature from room temperature to 85 °C increased the regularity of the crystals produced. It also found that regular particles could be produced, albeit at a lower level of regularity, without the addition of a chelate such as citric acid and this also substantially increased the yield. It was observed that mixing by shaking for a short time was simple to perform and yet still produced regular particles. The pH and temperature of the gel were also optimised in

the method to maximise regularity.

Investigation 2 tested if regular particles could be made in large quantities using precipitation. It was found that the hydrothermal method could be repeated with the same milder temperatures commonly used in the precipitation method and still produce particles with regularity. It was also possible to make regular particles of apatite without fluoride or a chelate added. Without the addition of these reagents, the yield was increased more than 10-fold. It was also found that heating the gel above 65 °C and cooling it for 10 minutes afterwards was the minimum required for crystals to form in the gel.

Investigation 3 tested if regular particles of apatite could be made in less than one hour. It was found that adding reagents together by pouring was simpler and faster than adding with a burette. Some mixing was necessary, but stirring for long periods of time was not. It was also found that using a microwave could produce the same particles of apatite as conventional heating on a hot plate, but in 10 minutes rather than in 4 hours. With further optimisation, this turnaround time was reduced to 15 minutes for 8 mL of gel.

Although this work was based on methods from the literature, it was uniquely focused on creating particles that were morphologically regular as well as tailorable. **It sought to optimise fabrication of these particles by combining and applying aspects of the methods in the literature that were considered optimal, an approach not undertaken previously for making regular particles.** Finally, it focused on the yield and turnaround time of the methods developed, which are aspects of apatite synthesis also rarely reported.

Thus the aim of the thesis was to optimise the conditions necessary to prepare a large amount of apatite with particles whose shape and size were uniform. Towards this aim the Author achieved the following. A method was developed that was substantially shorter than that reported elsewhere; one that could completed in less than quarter of an hour; the method produced particles of low crystalline hydroxyapatite with a minimal variance in morphology at a yield higher than 80%; and the procedure to make this precipitate was performed with equipment that was readily accessible. It

was found that the final microwave procedure developed was scalable; similar particles were produced when the reaction volume was increased 10-fold from 8 to 80 ml.

This work has sought to refine and to shift the current research focus from making small amounts of apatite particles with different shapes to making particles with tailored shapes in large quantities. It was hoped that some of the outcomes of this work will be of use in the construction of biocompatible monolithic materials for structural application within the body.

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Introduction

1.1 Making regular shaped and sized apatite particles with a simple method

The subject of this work was the fabrication of regular crystals of apatite for use as implantable material in the body, with a specific focus on the fabrication of fluorapatite and hydroxyapatite [1].

We claim and show that it was possible to synthesise with (a) less complexity regular particles of apatite [2] (b) to a greater yield [3] and using a method with a (c) shorter turnaround time [4] than reported elsewhere.

Access to uniform particles that differ little in size and shape was important. Without it, particles cannot be used in predictable ways and were certainly not pure. The

yield of the synthesis was also an important outcome, as without enough bulk material it was considered not possible to apply it to applications such as separation media [5] or for the formation of structural materials that are compatible with and for use in the body [6], which was the focus of this work.

The turnaround time of the synthesis was also considered crucial. Processes that take too long to complete, are the same as processes that have a low yield. A process that can be completed many times within the time it takes for a similar, but longer process, by default has the potential for greater output.

This work will also seek to make particles of apatite that have unique shapes. Most processes, some of which are listed in Table 6.1, were limited to making rod and sphere shaped particles. It was far more difficult to produce other shapes, especially if fluorapatite and hydroxyapatite, two common forms of apatite found in the body, are synthesised [1].

TABLE 1.1: Different methods of making apatite, make different shaped particles of apatite [7].

Shape	**Method**
Sphere	Mechanochemical dry reaction, Plasma spraying, **Wet chemical precipitation (see Section 2.4)**, **Wet Hydrothermal precipitation (see Section 2.3)**, Wet sol-gel
Rod	Mechanochemical dry reaction, Dry solid-state reaction, **Wet chemical precipitation (see Section 2.4)**, **Wet Hydrothermal precipitation (see Section 2.3)**, Wet sol-gels
Plate	**Wet chemical precipitation (see Section 2.4)**
Flower	**Wet Hydrothermal precipitation (see Section 2.3)**, Wet emulsion

Furthermore, this work will focus on the creation of particles that contain a microstructure internal to each particle. Reports elsewhere suggest that larger structures such as a centimetre-scale monoliths assembled from periodically assembled particles are tougher and, if the particles themselves also contain microstructure, this toughness

would be expected to be increased further [6, 8].

Most of the methods tested here were formulated from what was considered best practice in the literature [7], but with the addition of further improvements and insights developed in this work. However, many aspects of the methods described elsewhere were found to be unnecessary or became superseded by the findings described in this report.

With the optimization achieved, the final most optimized method (a complete description can be found in Chapter 6) used a domestic microwave oven (drawing on reports elsewhere [4]) and was tested and found to be useful for making large quantities of morphological uniform and highly crystalline apatite rapidly (within 10 minutes) to a yield higher than 80%.

There are caveats to the results in this work. The work focused on creating particles of regular morphology and many of the tests run failed to make these particles and so were not pursued further. With those tests that did make regular particles, analysis was not run if the yield was low. Also because of time, this work did not apply the particles produced herein to the creation of centimetre-size monoliths.

For those methods that did make regular particles with a high yield, analysis was not run in every instance if the regularity was obvious. It was reported in this work that if a 1:67:1 ratio of calcium and phosphate was mixed and uniform particles observed, only hydroxyapatite or fluorapatite (if fluoride was also added) was identified. Thus if this ratio was added, particle homogeneity could be used to indicate the presence of apatite.

To summarize, this work endeavoured to demonstrate that it is possible to make in less than 1 hour large quantities of particles that were uniform and mostly monophasic with reagents and equipment that was easily sourced. This work did not test if the particles produced could be assembled periodically into a monolith and if this morphological uniformity improved the mechanical properties of the bulk material [6, 9, 10]. This can be the focus of future work.

1.2 Current methods for making apatite and their limitations

Making apatite is a straightforward synthetic process. Simply add calcium and phosphate ions together in water [11]. If left on the bench this approach will form a pecipitate usually after a day since apatite has a low solubility. Many approaches reported previously have started with this simple approach, but then add further steps to this process for it to provide a specific end product.

For instance, changing the ratio of the amount of calcium phosphate ions that are added together from 1:1 to 2:1 calcium to phosphate changes the type of apatite that was made (as shown in Table 1.2). Adding another molecule, most commonly citric acid or ethylenediaminetetraacetic acid (EDTA), effects the way the apatite crystals are formed and the shape of the particles produced, and potentially how they might be used. Changing how this solution gel was heated can also have an effect on the precipitate. Gel heated to a high final temperature produced a precipitate that was highly crystalline [12, 13]. Those left on the bench to form, tended to produce particles that were amorphous [7].

TABLE 1.2: Common calcium phosphates are listed, each with a different ratio of calcium to phosphate and different properties [14].

Name	Ca:P ratio	pH stability	Space Group
Monocalcium phosphate monohydrate	0.5:1	0 to 2	Triclinic
Dicalcium phosphate dihydrate	1:1	2 to 6	Monoclinic
Octacalcium phosphate	1.33:1	5.5 to 7	Triclinic
Beta Tricalcium phosphate	1.5:1	NA	Rhombohedral
Hydroxyapatite (see Section 6.3)	**1.67:1**	**9.5 to 12**	**Hexagonal**
Fluorapatite (see Section 3.10	**1.67:1**	**9.5 to 12**	**Hexagonal**
Tetracalcium phosphate	2:1	NA	Monoclinic

Replacing the calcium, phosphate and hydroxide ions with other ions modifies the properties of the apatite. Exchanging hydroxide with a fluoride ion increases the crystallization of the precipitate. However, most of these exchanges reduce the crystallinity and thus the regularity of the particles, since most of these other ions do not exactly match the ions that they were replacing in size or charge and this disrupts the formation of the typical apatite crystal lattice.

The body does not contain pure phase hydroxyapatite ($Ca_5(PO_4)^3(OH)$). For the most part, this apatite with many ion substitutions. Carbonated hydrogen apatite is probably the most common variant in bone whereas fluorapatite ($Ca_5(PO_4)^3(F)$) is common in the thin outer of enamel and provides teeth with a hardening property [5]. In addition, many of the implantable materials used in bone restoration rely on amorphous forms of hydroxyapatite rather than crystalline hydroxyapatite itself, as these were observed to more likely trigger bone regeneration. They best mimic the precursor forms of apatite rather than the final form found in bone.

An important objective in this work was the production of regular apatite particles. Irregular particles by their nature have irregular properties. Regular particles are more likely than not to have predictable properties [5]. They were usually monophasic, they do not contain different types of apatite, which is an important attribute for producing artificial material with predictable properties [4]. This regularity also allows close packing of particles in a regular manner which is considered necessary if the bulk material produced has the desired mechanical properties.

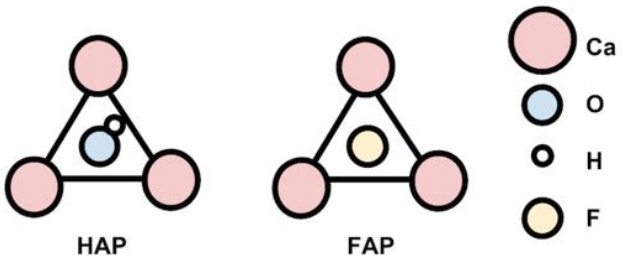


Figure 1.1: A partial crystal lattice of hydroxyapatite (HAP) and fluorapatite (FAP) (the model shown is not proportional). Under the same conditions, particles of fluorapatite have a crystallinity and complexity to their morphology that is greater than particles of hydroxyapatite [15].

In this work the focus will be mostly on the fabrication of fluorapatite and hydroxyapatite particles. The difference between the two is that the fluorapatite contains a fluoride ion and hydroxyapatite a hydroxyl ion (as illustrated in Fig. 1.1). Apatite particles that contain fluoride are usually also more regular in shape and have a higher crystalline structure and are thus harder than their hydroxyl apatite counterpart. There is a trade-off here as they can also be more brittle.

The various methods described in the literature for fabricating apatite are well defined. As mentioned, apatite can be made simply by mixing calcium and phosphate together and leaving the gel that forms on a bench for particles to precipitate [7]. However, this simple approach typically makes particles that are small, irregular and spherical in morphology and with a low crystallinity. Its incubation can take several days to complete and thus the particles produced this way are most likely inadequate for some applications, such as a source material for a high-strength bone implants.

There are a number of systematic reviews on the topic of the synthesis of apatite and in particular on the topic of the effect that different synthesis methods have on the size of the particles produced and the shape that are made [7]. These articles show that particular approaches to the synthesis of apatite produce particular particle morphologies. The most significant factor that determines the shape of and size of the particle is the type and number of reactants, other than calcium and phosphate sources, in the reaction mix. To a lesser extent, particle morphology is dependent on the gel's pH and its final incubation temperature. In addition, specific procedures for making apatite, such as the flame and emulsion methods, tend to produce spheroids no matter what reaction conditions are set. For further discussion on this topic, the reader is referred to the following excellent review articles [7, 16].

Apart from precipitation, there were alternate procedures reported elsewhere that improve the quality of the particles produced [7]. For instance, the hydrothermal method has been shown to produce regular particles whose shape can be manipulated [17]. This approach uses a pressurized vessel and heats to a high temperature above the boiling point of water and includes the addition of chelating chemicals to further control the fabrication of the particles, such as citric acid. Because of the use of this

apparatus and the high temperature of incubation, the procedure was more involved than the precipitation approach and requires specialised equipment. It can also take some time for the incubation of the solution gel to be completed.

In an attempt to repeat the benefits of the hydrothermal approach and combine this with the simplicity of the precipitation approach, a microwave approach which uses dielectric heating instead of conventional thermal dissipation of hot plates for the precipitation or ovens for the hydrothermal has been reported [7]. It was demonstrated that high quality particles with sophisticated shapes can be produced rapidly with this approach. Additionally, the microwave does not have to be specialised laboratory apparatus. The procedure can be performed with a domestic machine, albeit with less control, as a domestic device does not have sophisticated control over heating and setting to a final temperature [18, 19].

The methods tested and further developed in this work were based on those already cited in the literature [7]. Its novelty comes from its attempt to combine together into a single method the best of aspects of these earlier approaches to further shorten and simplify the fabrication of apatite without compromising the morphology of the particles formed, and in particular the formation of an anisometric-shaped particles that are regular, which was already cited as a particular aim of this work [1, 6].

Some reports elsewhere demonstrated the use of calcium nitrate tetrahydrate and diammonium hydrogen phosphate reagents in water for the formation of apatite [7]. The advantage of these particular reagents, and the reason they were applied in this work, is that they have a high solubility in water and are readily obtained. After their combination and mixing, the pH of their gel can be adjusted with nitric acid and ammonium hydroxide and this provides a further advantage, as these reagents do not further complicate the gel with additional reagents, thus keeping the components and end products of the reaction gel simple and producing regular particles and its analysis the focus.

An additional focus of this work was the amount of precipitate produced. Implantable materials typically require gram-scale amounts. To achieve this yield, this work tested reactions concentrations at at sub-molar to molar levels rather than the

milli-molar concentrations typically reported elsewhere [19]. Other changes to the methods used by reports elsewhere were made. Usually a low pH was set for the hydrothermal synthesis (around 6 to 8 pH) and a high for the precipitation and microwave synthesis (around 9 to 11 pH) and then maintained [20]. However, in this work the pH was set, but then allowed to self-adjust as the reaction proceeded. This self-adjustment was usually involved a drop in the pH of the gel and is described in more detail in Section 6.8.

Most previous work elsewhere controlled how the calcium and phosphate reagents were added together and then mixed or did not report this aspect of the method [20]. It was quite common for reports elsewhere to describe slowly adding reagents together using a buret and then to continue to mix the formed gel with a stirrer bar as the reaction proceeded. It was also quite common to perform this initial pouring and mixing at room temperature [19], and then heat the gel to allow the reaction to proceed at a faster rate so that it could be completed in single laboratory session rather than having to leave it to run overnight [10, 21]. There were no reports, as far as the Author is aware, where the two reagents were simply poured into each another and then mixed by shaking, an approach that was used in this work.

The temperature of the solution gel in this work and in the literature was run at a temperature below boiling point if a precipitation or a microwave method was used, or it was heated to above boiling point if a hydrothermal approach was used [17]. In almost all examples in the literature, however after initial heating, the incubation temperature was then maintained for its entire length. The slow heating and cooling of the gel used here was not reported elsewhere or was not considered an important aspect that required control [7]. There was only one example reported elsewhere, as far at the Author is aware, where this was the focus of the work [21].

It was common to the literature, and the approach taken here, for the precipitate produced, to then be washed [7]. This washing step could involve the separation of the supernatant from the precipitate by centrifuging. It could also be performed with a filter system that removed the supernatant by washing the precipitate through. In almost all cases in the literature, the precipitate was washed with water, and occasionally

with a mixture of water and ethanol, possibly to reduce washing time [7]. In almost all reports elsewhere, the precipitate was washed at least twice and usually three times [19].

After this washing treatment, as reported elsewhere [7], the precipitate was then usually dried in an oven to produce a fine white powder. Very rarely was the precipitate held in solution for further application. For most uses, a simple drying step was described. However, for some uses such as the intended assembly of the particles in this work where uncontrolled aggregation of the precipitate should be avoided as it can introduce defects, maintaining the precipitate in water after washing was considered necessary.

Most of the literature methods for the production of apatite ran for a few hours or overnight [7]. The shortest time possible for the simple precipitation method using a hot plate for heating was approximately 4 hours and a similar time for the hydrothermal method was also reported [7]. If the temperature of the solution it was incubated to was low, the reaction would proceed for a few days until its completion. The microwave method in contrast was rarely reported running for longer than an hour and was usually completed within half an hour [19].

As can be seen from this discussion, there are many ways to make apatite. It can be simple and quick if a precipitation approach was taken [13], but the particles produced from this method were usually irregular and small [10]. Much larger and more regular particles can be made with procedures such as a hydrothermal method [17], but this approach can be complex to run, can take much longer to complete and can have a lower yield, especially if a chelate was added. Since larger more complex crystals produced at a high yield was quite useful [22] what appears to be lacking in literature accounts was a method that can do both; a method that was simple and quick to complete and yet could make large amounts of crystals that were regular and could be tailored.

Therefore a position that could be drawn from the literature and the focus of this work, was that the methods for making a apatite are not yet fully optimised for the formation of large quantities of regular particles. Here we hoped to develop such a

method that was a combination of the best of the hydrothermal, precipitation and microwave methods as well as having included new insights and observations drawn from our own testing. We hoped that this novel and optimized method would make it feasible to create regular particles to a high yield quickly.

1.3 Examples of artificial materials that combine biocompatibility with high strength

Bone and teeth that are in disrepair, can be restored with the addition of materials that are typically made rather than sourced from nature [23]. The properties of these materials usually were a compromise between their compatibility with the body and their mechanical strength [6]. The compatibility of these materials varies; some materials can be completely absorbed when inserted into the body, others are not toxic, but remain inert. Their strength also varies; some are strong in compression, some in tension and some are strong in shear.

Unfortunately, artificial materials rarely combine biocompatibility with a high-strength. For example, apatite is compatible with the body, it can chemically interact with body fluids, but in its pure form is hard and brittle [22]. Materials made from metals are usually non-compatible in the sense that they can remain inert and cannot be broken down by the body, but they are also strong, pliable and tough. Thus the application in the body of materials containing apatite was usually limited to non-load bearing uses [24]. Whereas the application of metal based materials were limited to applications where their inertness was not harmful [22]. In some instances, apatite and metals were combined to make a composite, but this material was a compromise of compatibility and strength and did not fully satisfy either [6, 23].

This work aimed to develop methods for fabricating crystals of apatite that potentially could be assembled into composites that might then mimic the structures found in bone and teeth. To do this, it was organised into Chapters that described the methods used to make these particles, the results obtained and a discussion of how they

compared to the literature and finally a summary of the findings unique to this work.

1.4 Examples of artificial materials that can aid in restoration and have a long working lifetime

It is rare not to suffer some amount of tooth decay. Without its removal and replacement with restorative material - whether it is amalgam, resin composite or glass-ionomer - the tooth can be lost and oral functionality compromised [1]. But with attachment of restorative material some of the natural tooth material unaffected by the disease is also lost.

In turn, the new material added has a limited working life and at some point will fail and have to be restored again with new material, which leads to further loss of intact natural tooth [1]. It is not uncommon for the cycle to repeat itself a few times and eventually for most of the original material of the tooth to be lost and for the original tooth to then be replaced with a crown or a denture made of non-biocompatible metal or ceramic.

Likewise, it is not uncommon to suffer some loss of joint functionality later in life. Replacement with artificial materials can prolong the patient's ambulatory function [23], but these also have limits to their useful working life. Fortunately, since these replacements are usually done late in life, a finite working life is typically not an issue.

However, and much like restored teeth, the replaced joint can sometimes experience a complication, and this can lead to early joint failure and the need for its replacement [23]. So it would be of interest if a restorative material could be applied that reduced the removal of non-diseased tissue in the body, because it was made from material the same as or similar to what it was replacing.

1.5 The challenge of making centimeter-sized artificial materials that contain a periodic nano-scale microstructure

A large number of reports have attempted to make bone or tooth analogues that combine the biocompatibility and toughness found in their natural counterparts [25, 26]. These analogues usually incorporate two materials as a composite. One material is usually strong and hard, the other soft and tough [8]. Most of these synthesis attempts have focused on bone analogues [6] with less progress on teeth [1, 27], if the progress in the attempt to grow new teeth within host tissue are ignored.

So far none of these attempts have met with good clinical outcomes and this might be due to their failure to recreate the complex microstructures found within their natural counterparts [23]. These structures can be described as multilevel, hierarchical and having a brick-and-mortar structure. The microstructure occurs periodically and anisotropically throughout the material [11], and artificially reproducing these nanostructures across centimetre-sized material has proved to be quite daunting [1].

The structure of nacre is shown in Fig. 1.2 [11]. A series of isotropic plates are stacked one on top of the other (in a brick and mortar pattern), separated by organic matter. Its four features, a (i) periodic (ii) stacking of (iii) regular particles with an (iv) inter-particle fugitive interface provide its unusual mechanical properties. Nacre has a high compressive strength (540 MPa) with a low tensile strength (5 MPa) orthogonal to the stack, and a high compressive (235 MPa) and high tensile strength (170 MPa) parallel to the stack [24]. Tooth has a similar arrangement and response to load, and this is illustrated in Fig. 1.2 (b) [11]. It was envisaged that a useful biocompatible artificial bone or tooth would display a similar structure [6, 23].

How these microstructures enhance the mechanical properties of a material is of interest. A material's microstructure can refer to an assembly of small units that make up and form a larger structure, whether these units are sub-micron sized particles that form a centimetre-sized monolith or smaller nano sized particles that form the same

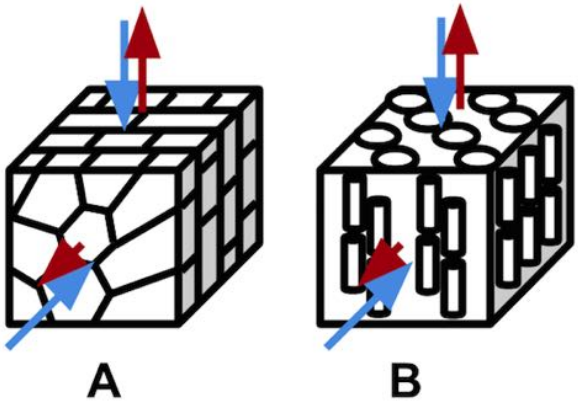


Figure 1.2: The importance of microstructure is illustrated by a representation of the microstructure found in shellfish (A) and one for enamel (B). The blue arrows represent a compressive load and the red a tensile load. The length of each arrow, which is indicative only, shows the capacity for each structure to carry these loads till failure. As can be seen, each of these materials can support a much higher tensile load parallel rather than perpendicular to its microstructure. If a microstructure was not present or present but was not unidirectional, the tensile load shown would be lower and the same across each axis [6].

sub-micron sized particles [28].

The Author notes that in many reports elsewhere, the particles of apatite produced and shown are at the several hundred nanometer to several micrometers in scale [9, 23, 26]. Rarely are particles produced that are less than 50 nm or more than 5 microns in scale and these smaller particles are usually fabricated without simple mixing and without heating and so are amorphous and non-crystalline; they are usually made from non-apatite calcium phosphate. This amorphous material can have potential some applications such as in enamel remineralisation, but less so in tissue engineering, which requires structural strength and so was considered outside the focus of this work. The larger multiple-micron sized particles were also not considered since reports elsewhere suggest that these larger particles are similarly structurally weak and can take time to grow, and so can be more difficult to control. Wide-spread reporting elsewhere of apatite particles at the micron-scale suggests that this scale of particle is of greater interest and use [28].

A microstructure can also be thought of as a regular array of nano spaced micro-cracks or flaws. Ironically, it is the presence of this periodic fine cracking that can provide a material with a greater resistance to cracking when placed under load [6].

The load is more likely to be spread rather than focused and the failure of the material to occur piecemeal within these fine cracks rather than catastrophically at single macro-defect [6].

The methods used to make analogues of bone and teeth can be grouped into two (misleadingly classified as most do both) approaches: (a) top-down or (b) bottom-up. The top-down approach takes a block of material and processes this material to create a microstructure within it. The material itself provides strength and the microstructure formed within, its toughness [9, 23, 26].

In the bottom-up approach the opposite occurs. It takes nano-sized particles and assembles them into a block of material. The assemblage provides the strength and the microstructure formed from the interface between the particles, provides the assembled material its toughness [7].

Most attempts in the literature have had mixed results as it was difficult to make a periodic microstructure across centimetre-sized material. Natural examples give some clues as to what a potential artificially made solid monolith of particles with a high toughness might look like. Such a material would contain (1) uniform mineralised particles, (2) that are anisometric and aligned, and (3) closely packed in a nematic fashion and as well (4) contain material between each particle that was elastic.

Furthermore, these particles would be made from either an apatite or a carbonate, or be made from a material that could be transformed into either of these, as these materials form a major component of bone and teeth in animals and as such are compatible with and readily absorbed by the body. Some of these structural attributes are shown in Fig. 1.2.

In this work, an approach of assembling particles into a bulk material was assumed to be more practical than the creation of a microstructure in bulk material [6, 28]. These particles would also be anisometric so that they could be packed and aligned periodically to mimic the unidirectional microstructure seen in nature, and as illustrated in Fig. 1.2 [6, 8].

In summary, it would appear that a bottom-up rather than a top-down approach is

more likely to achieve sought after goals of this work. It was considered more practical, although not easy, to make bulk amounts of regular particles. Apatite was preferable to carbonates since there was already support for their synthesis as regular anisometric particles in the literature; their generic protocols are listed in Table 6.1. The shape that apatites commonly form are unidirectional. Fluorapatite and hydroxyapatite found in bone and teeth are formed from the hexagonal arrangement of their ions which create rod or plate shaped particles, and this is illustrated in Fig. 1.3.

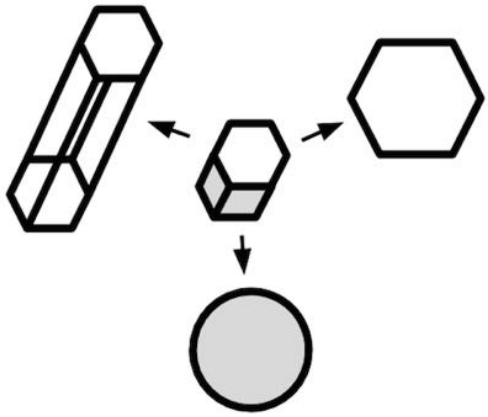


Figure 1.3: The ions that bind together to form apatite do so via a hexagonal crystal packing order and these in turn can further agglomerate to also form hexagonal shape nanoparticles as well as sphere and rod-shaped particles [29, 30].

These particles can be obtained from natural sources or artificially in the laboratory, as shown in Table 1.3. It appears, however that fabrication of artificial apatites rather than their acquisition from nature would be more straightforward. Particles that are made rather than acquired are more likely to be tailorable and have a higher purity. The hydrothermal, precipitate, microwave methods appear different, but it should be kept in mind that they only differ in the conditions of how the solution gel was heated [7]. An issue with making this material however, and one addressed in this work, is if these particles could be made in bulk amounts, and this is the focus of the next section.

Accordingly, this work is based on the assumptions outlined here. **That is, that very strong tough solid non-porous cm-scale materials (referred to in this work as monoliths) require (1) particles that were fabricated rather than sourced from nature and that these (2) particles are biocompatible and (3)**

TABLE 1.3: Natural and artificial sources of apatite.

Source	Method
Animal	Calcination
Plant	Extraction
Precursor chemicals	Dry solid-state reaction
	Mechanochemical dry reaction
	Plasma spraying
	Wet chemical precipitation (see Section 2.4)
	Wet Hydrothermal precipitation (see Section 2.3)
	Wet sol-gel
	Wet emulsion

regular in size and anisometric in shape [6, 28]. It was thus assumed in this work that the first step towards making tough monoliths was the fabrication of particles from which it could be formed, and this was the subject of this work. The actual assembly of the particles to form a monolith and the mechanical testing of the monolith was not covered in this work, but reports elsewhere provide some indication as to how it might be made possible [10]. It should be pointed out that the assumption given here that a potential solution to the problem given was particle-based, however may not be correct. Even if it is proven incorrect, the approach taken here has the advantage of being direct and might still be useful in informing other attempts at the fabrication of bone and tooth analogues.

A major focus of this work is to produce a precipitate that is made of particle micron in size and uniform in the distribution of its size and shape. For the purposes of consistent terminology, the Author will refer to uniform particles as "regular" and non-uniform as "irregular". In this work, regular particles are particles that have the same type of shape i.e. the precipitate does not contain rod and sphere shaped particles in the same mix, and the particles have a similar size i.e. the percipient

does not contain particles half of which are micron and half of which are nano in size, but have a majority (at least 95%) of one size or the other. In same samples, these regular particles are seen in aggregates that are irregular. This aggregation of particles is considered in this work, and can be seen in reports elsewhere, as a function of the conditions of their drying in preparation for visualisation usually with an SEM and so was not considered of import. When the particles in water were measured with a Malvern Zetasizer, this effect was minor or not observed and this suggests that it was an artefact of drying. It was also one of the intended outcomes of this work that it might lead to the fabrication of apatite for use as a bio-compatible cement in injectable material. As such it would be formed and kept wet and not allowed to dry so that the aggregation observed would be minimal.

1.6 A simple high-yield method for producing regular particles

The synthesis of apatite is well-documented and understood and, apart from incremental development, it is hard to imagine new insights. There might, however still be opportunities for improvement. One such opportunity could be drawn from the amalgamation of the best practices cited in the literature. Another may come from repeating the best practice and the serendipitous observations during this repetition. Finally, opportunities may arise from how the procedures described in the literature could be modified for their practical application in industry. In this regard the focus of the procedures is less about the development of a new types of particles, and more about the development of procedures that can produce the desired already observed particles, but in a more efficient manner.

While apatite production in industry are routinely employed, having a better approach to this fabrication - an approach that is robust, repeatable and straightforward - would still be valuable and could also provide basic research in the laboratory with a synthesis standard that would allow the researcher to focus on potentially useful

fundamental and applied applications of apatite rather than spending time optimising its synthesis. Thus this work focuses on the development of and the judgement of a procedure that reduces it's turnaround time by about a factor of 10, the complexity of the solution gel by a factor of two, and the complexity of handling in the procedure, so that apart from the mixing and heating step, no further handling is required.

Given the preceding discussion, some conclusions can be made and plans formulated. The precipitation method given in the literature was simple to run and produced to a high yield; the reagents can be added together and left on a bench to incubate [31]. The method, however could take days to complete [10] and the particles produced were typically irregular spheres, which indicated they had a low purity and crystallinity, and the particles made were simple and contained no internal structure.

The conventional hydrothermal method typically used a pressure vessel to heat the gel above boiling point and additives such as citric acid or EDTA to provide control over the morphology of the particles produced. The method can fabricate particles that were highly regular [32], some containing an internal assemblage of smaller particles, but the method could also be complex to run and had a yield that was low [32].

It would be better if the advantages of both of these methods could be combined and a new method developed that was simple to run, had a high yield and could produce regular particles with a complex internal structure. To achieve this, in this work the optimal aspects of methods in the literature, as well as the development of new approaches not seen before, were identified and then combined into a single efficient method [32].

To implement this approach, the work tested a hydrothermal method with a focus on increasing its yield and reducing its turnaround time [16, 18]. When this was not achieved, this work then tested a precipitation method and applied the lessons learnt from the hydrothermal tests to this new approach [3]. When this too failed to produce a practical result, some of its more useful aspects were then applied to a method that used a microwave in an attempt to keep the length of the method as short as possible.

Any aspect of these three approaches tested were considered useful when they produced a precipitate of uniform single-phase anisometric crystals of apatite that was

nano or micron in size or had a yield at least 50% or higher [3] or was able to be completed in less than one hour [18].

Literature and Patents reported elsewhere in the field of bone cement for tissue engineering made with apatite suggest that a particle size of about 1 micron as a suitable size for the main filler in injectable material that hardens once placed in the body [33, 34]. Filler made from particles at sizes above this tend to have a viscosity that is to high for injection, those at sizes below a micron tend to be less crystalline and are thus less able to resist a mechanical load or the depletion of ions in fluids and could loss their integrity once placed in the body [35].

1.7 The objectives of the thesis

An assumption of this work, was that where apatite fabrication was reported elsewhere, it was not fully optimized for the formation of uniform and monophasic apatite. It does not appear that the different conditions described as necessary were bought together and tested in a single work [7, 16]. The Author was not aware of a report elsewhere that combined and applied most aspects of apatite synthesis considered best practise in the literature.

This work planned and then repeated those aspects of methods reported before and that was highlighted in this review and that was considered best practise in the literature or optimal by the Author. This work anticipated minimizing or removing steps reported elsewhere that appeared to not contribute to the formation of regular particles. In some sections, this work tested new approaches to making apatite and then incorporated those results which were most promising into a final and optimal method.

The thesis itself is structured as follows. It first tested aspects of the conventional hydrothermal methods reported elsewhere (the final method is shown in Section 2.3) as this approach was known to produce regular particles [30, 36]. It then repeated and extended the precipitation approach (a typical method is listed in Section 2.4), although it had not shown that it could produce regular particles, this approach was

simple to run and produced a high yield precipitate [35].

With both of these approaches, this work attempted to reduce the overall complexity of the make up of the reaction gel (also referred to in this work as the solution gel) [32]. **It was thought in this work that the addition of less reagents to the initial reaction would simplify the synthesis tested as well as possibly improve the yield and provide greater control over the morphology of the particles produced and their analysis.**

An additional aim of this work was to shorten the overall time required to make apatite without a losing or reducing the quality of the precipitate produced. In this aspect, the work applied a microwave method, which was reported elsewhere, for heating of the gel (the optimized protocol is listed in Section 2.5) as this was thought to greatly reduce the length of the synthesis [13, 19]

This work also tested if crystals could be made as large as possible and if it could contain a complex internal microstructure. As suggested, including an internal microstructure might provide the particles produced with enhanced mechanical and chemical properties. Reports elsewhere indicate that a method that can make a larger rather than a smaller particle, would have a greater chance at optimization [14]. A method that makes only smaller particles would provide less opportunity to generate different morphologies.

In summary, this work aimed to develop (1) a method that made regular-morphology crystals of apatite, that was also simple to run; (2) a method that produced particles at a high yield that were uniform; and to develop (3) a method to make crystals in a short time that were as large as possible.

2

Method

This Chapter lists the three methods for making apatite tested in the course of this work. There are other methods for making apatite [7], but these were not tested if they were considered by the Author to be too involved, or unlikely to produce the precipitate that was wanted. Further iterations of the methods are reported in Chapter 3, which extends the conventional hydrothermal method, and in Chapter 4, which presents novel hydrothermal treatments, and in Chapter 5, which demonstrates an optimised precipitation method, which was then further developed in Chapter 6 as a rapid microwave method.

The features common to all of these procedures are listed in Section 2.1. Features specific to the hydrothermal method are listed in Section 2.3, features specific to precipitation are listed in Section 2.4 and those for the microwave are listed in Section 2.5.

The three methods share features: each adds phosphate and custom

solutions together, each incubates the gel and each washes the precipitate that was formed. The hydrothermal method, shown in Section 2.3, however differs from the other methods in that it heats the gel above boiling point inside a pressure vessel using an oven. The precipitation method, described in Section 2.4, differs from the other two methods in that it heats and incubates under atmospheric conditions. The microwave method, described in Section 2.5, repeats much of the precipitation method, except the gel was heated with a microwave oven and not with a hot plate or for that matter a conventional oven.

These methods also share many of the features found elsewhere in the literature [14, 34]. A major difference, however between what was reported elsewhere and this work, was that the methods tested here combined the conditions considered optimal elsewhere into each of the three procedures tested and these procedures included the addition of further insights uncovered through the tests performed as part of the work described here.

Unless otherwise stated by the Author, the choice of reagents chosen are based on existing and accepted synthesis procedures. Where they are not, the Author has added appropriate information on the choices of process parameters and reagents as part of the discussion in each experimental section that are listed in the next Chapters. There is also further discussion on the choices of process parameters made for the final and most efficient methods tested and improved upon in this work, the microwave method which is described in Chapter 6. The microwave method is also listed as a draft of a journal paper and provided in Appendix A.

Where the word crystallinity is used in the text of this work, this term refers to the level of the packing structure of the particles formed and this can be seen in X-ray diffraction (XRD) pattern as sharper peaks. The more crystalline a precipitate was, the more regular its atom packing structure, the denser and more resistant to dispersion it is and the sharper its form appeared in the SEM. The definition of the term geometrical shape of particles used in this work is related to the term crystallinity, but is considered to refer to a different type of property. In most samples, crystals with defined prisms are generally more crystalline than those that are not. Particles that

appear to have no defined prismatic structure are unlikely to have ordered packing or have sharp peaks in their XRD characterisation.

Precipitates that have acceptable regularity are those that have clearly defined particles, particles that are similar in shape and size, and form precipitates that appear to contain no other visible material apart from these particles.

2.1 A general method for making apatite

Apatite can be made in a single step by mixing together a phosphate and a calcium solution and then leaving it to gel on the bench long enough for a precipitate to form [10].

To impart a desired property onto the precipitate, most methods described in the literature were more involved than this and most used equipment and reagents of calcium and phosphate that were easily acquired and straightforward to apply [7].

For most, the solution gel was first prepared by adding the reagents together, mixing them well, and then incubating the gel at a low temperature, usually room temperature [10], or at a high-temperature, usually between 60 and 200 °C [36]. The gel was prepared with [32, 34] or without [19] the addition of extra reagents that did not contain calcium or phosphate, to provide particular properties that were desired in the precipitate produced.

The general procedure listed in Fig. 2.1, was followed by the three methods tested and described in this work. For each of the three methods, the reagents used were prepared and applied at the same concentrations so that the results for each methods could be compared to the other two methods. The concentrations tested were between 20 and 200 mM for the calcium reagent. The other reagents tested were then mixed together at the same concentration as the calcium, but at different volumes, to form the gel; usually at a ratio of 5:3:1:2 for the calcium, phosphate, and (if it was tested) fluoride and citric acid (or EDTA) reagents.

The ratio of calcium to phosphate described here that was added to the gel was

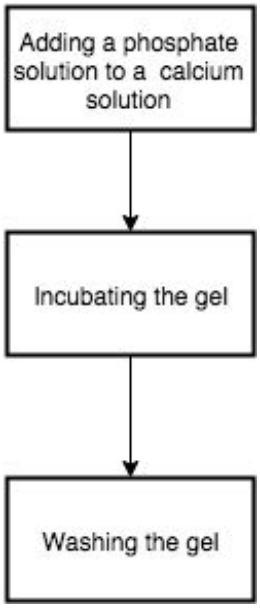


FIGURE 2.1: The core steps used to make calcium phosphate precipitate is shown. The hydrothermal, precipitation and microwave methods described in the literature and tested in the course of this work shared these common steps.

1.67:1, which is listed in Table 1.2 as the ratio found in hydroxyapatite and in fluorapatite. This ratio could be changed slightly and was found to still produce mono-phasic hydroxyapatite or fluorapatite precipitate. If the ratio added differed more than about 5% from 1.67:1 (data not shown), it was found that the precipitate contained hydroxyapatite or fluorapatite and at least one other phase listed in Table 1.2.

2.2 The methods used for the analysis of the reaction precipitate

After the reaction was completed, to calculate the yield of the precipitant, the product was prepared by filtering it using a number five paper under a vacuum pump until a dry powder was formed. It was then rinsed in approximately 100 mL of distilled water and then 50 mL of ethanol at 95% and dried by suction. It was then further dried for two hours at 60 °C under a moderate vacuum at approximately 50 mm Hg of pressure. About 1% of the total mass of the precipitate was assumed to be lost during

its handling since traces of it were seen on the filter paper.

The precipitate was also washed and dried before a series of analysis were performed for its identification. For analysis by energy-dispersive X-ray (EDX) spectroscopy, it was washed with ethanol, left to dry and then imaged with an Hitachi SU-70 field emission scanning electron microscope set at a 7 kV acceleration. For X-ray diffraction (XRD) analysis, the precipitate, prepared the same way, was scanned with a D2 PHASER (Bruker) diffractometer. For Raman analysis, the similarly prepared precipitate was analyzed with a Renishaw inVia microscope that used a near infrared 785 nm laser.

A thermogravimetric analysis (TGA) Setaram LABSYS evo system was used on the precipitates produced in each method tested to confirm the purity of the identified fluorapatite (Fig. 3.10) and the hydroxyapatite (Fig. A.1) precipitate. The dried precipitate was heated at 10 °C a minute to 1200 °C in nitrogen. A scanning electron microscope (SEM) set to 1.5 kV acceleration was used to image the crystals across a 2.5 to 40 K magnification range after each test. For this imaging, wet precipitate was first pipetted onto Al stubs and dried. A Malvern Nano ZS Zetasizer measured the particle size of the precipitate made with each method. For this test, the precipitate was kept suspended in water set to a neutral pH after first washing it, but not drying it.

Statistical analysis of the dimensions of the particles seen in the SEM images was performed using ImageJ 1.48 (by NIH) and SPSS 22 (by IBM) software. To perform this analysis, the original TIFF images were converted to 8-bit binary by ImageJ and then measured using a plugin (Cell Magic Wand by Theo Walker). These measurements were analysed using a one-sample t-test to calculate the mean and its 95% confidence interval (n = 30). A one-way ANOVA and a Tukey post-hoc test were performed to test if the difference in crystal sizes seen for the different synthesis methods tested were significant. The normality of these tests were confirmed with Kolmogorov-Smirnov and Shapiro-Wilk analysis of the data.

2.3 A hydrothermal method for making apatite particles

The hydrothermal treatment heated the solution gel, enclosed in a pressure vessel, above boiling point over several hours. To do this, a 300 mL calorimeter stainless steel vessel (Parr Instrument Company) was re-purposed with a Teflon lining. The gel could be added and safely heated to 200 °C in this enclosed container [33, 34]. A gas chromatography oven was repurposed to heat the vessel. It was either preheated before adding the vessel or the vessel was added and then it was heated slowly from room temperature to a target temperature.

For preparing the solution gel, 1 M working solutions were prepared from doubly distilled water of the reagents as they were received. Calcium nitrate tetrahydrate was used instead of calcium chloride, even though the latter produced more regular particles, as its use would also introduce chloride into the gel and this would further increase in number the elemental composition of the gel. Likewise, 1 M working solutions were prepared of citric and ethylenediaminetetraacetic acid (EDTA) as received from double distilled water [33, 34].

Likewise, when fluorapatite was made sodium in the gel was avoided if ammonium fluoride was added instead of the more commonly reported sodium fluoride. There was no observed difference in the crystals produced with the addition of either reagent. Phosphoric acid was also tested as a phosphate source instead of dihydrogen ammonium phosphate and it did produce crystals, but these were observed to be less regular (data not shown) and so its use was not continued. Preparations of other reagents used in one-off tests were similar to the way those listed here were prepared and details of their preparation are given where their use is described in this work.

Other reagent mixes, such as chelating agents, were also tested and this is also shown in Fig. 2.2. These additives were dissolved in the phosphate solution and before the calcium solution was added. Once the gel was prepared, the pressure vessel was sealed, placed in the preheated or soon to be heated oven and the gel left to incubate, typically for 4 hours. Once incubation was complete, the hot vessel was removed and

immediately place in a water bath at room temperature to cool, which usually took 10 minutes.

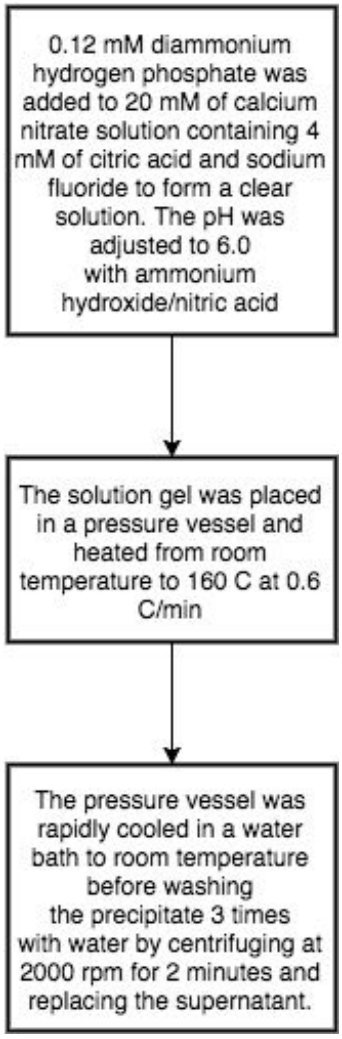


FIGURE 2.2: The typical hydrothermal method used in this work for making apatite is shown [33, 34].

The reagents were prepared to the same concentration, but were added to the solution gel at different volumes. The ratio of these volumes as mentioned were typically 5:3:1 for the calcium, phosphate and fluoride reagents. Citric acid was added at a ratio of 2 and EDTA was typically added at a ratio of 1. The concentrations of the solutions tested ranged between 20 and 200 mM.

The solution gel was heated to between 80 to 200 °C. The length of time of this

incubation varied between 1 to 16 hours. Particles were washed after each incubation three times in double distilled water, before they were stored still suspended in water so that their tendency to self-aggregate could be minimized. They were then prepared for analysis as described previously. If ethanol was used instead of water for washing, the particles were observed to sediment (which suggested some aggregation) and dry quicker.

2.4 A precipitation method for making apatite

For the precipitation treatment, the procedure was similar to that described in the literature and was found to be simpler to implement than the hydrothermal method [13, 35]. It differed somewhat from the hydrothermal and microwave procedure. Instead of a pressure vessel, an open glass bottle with a screw cap was used and its procedure is listed in Fig. 2.3. The solution gel was heated over several hours on a hot plate. In a typical experiment, the phosphate solution was added to the calcium solution either fast by pouring it or slowly by dripping it from a burette [16].

The reagents used were the same as those described in the hydrothermal method, except fluoride and the chelates were omitted so that an unchelated hydroxyapatite at a higher yield could be made. Typically, in this procedure the calcium solution was added at concentrations of 20 to 320 mM. The gel was heated from room temperature on a preheated hot plate set to a temperature between ambient and 85 °C [3]. The gel was left to incubate on the hot plate for 1 to 8 hours. The gels tested were either left unmixed or mixed with a stirrer while they were incubated. Like the other two methods reported in this work, the precipitate produced were then washed with water three times.

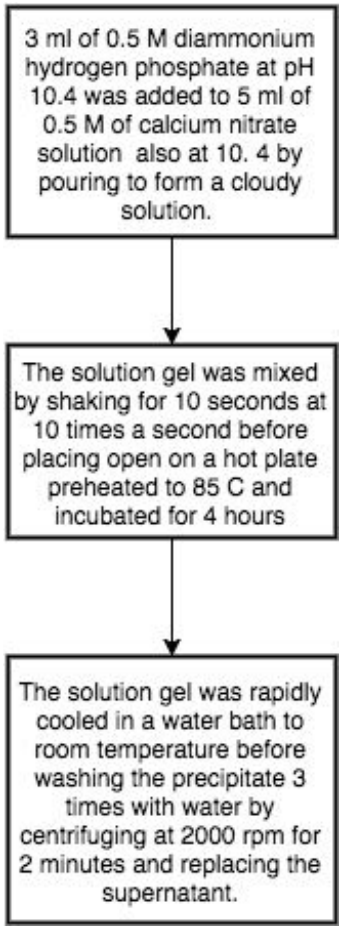


FIGURE 2.3: The typical precipitation methods used in this work to make calcium phosphate.

2.5 A microwave method for making apatite particles

The initial method was similar to the precipitation method, but used a domestic microwave (Homemaker EM820CTM, 800 watts) to heat the solution gel. The reagents prepared were also the same as those used in the precipitation method. As this work progressed, the microwave method was refined to the final one listed in Chapter 6.

Volumes of up to 400 mL could be processed at once if a domestic microwave was used, but there was minimal control over how this gel was heated. Typically the simplest approach was to run the microwave at full power and heat the gel at

a rate determined by the volume that was heated. The speed of heating could be slowed by adding containers of water along with the gel. Upon reaching a target temperature (predetermined by heating the same volume of water and then measuring its temperature), the microwave oven was switched off and the sample removed and left on the bench to cool. For an 8 mL volume of gel, 14 seconds at full power was needed to raise its temperature to 85 °C [3]. For an 80 mL volume of gel, 1 minute and 20 seconds of microwaving was needed to raise its temperature to 85 °C.

As shown in Fig. 2.4, in a typical test precursor reagents were added to a glass bottle, the bottle was sealed and the gel mixed by shaking with a mechanical shaker or by hand. The bottle left open was then placed in a microwave oven and heated before its removal and incubation, without further agitation, on a bench to cool. Like the other two methods reported in this work, the precipitate that was formed was washed with water.

2.6 A summary of the methods used in this work

In summary, three methods for making apatite were further developed by the Author - a hydrothermal, precipitate and a microwave method - and their procedures were listed in this Chapter. These methods were based upon an amalgamation of reported conditions described as optimal by the literature and new insights uncovered by tests described in this work. The results of tests that used a hydrothermal approach listed in Section 2.3, are reported next in Chapter 3 and in Chapter 4.

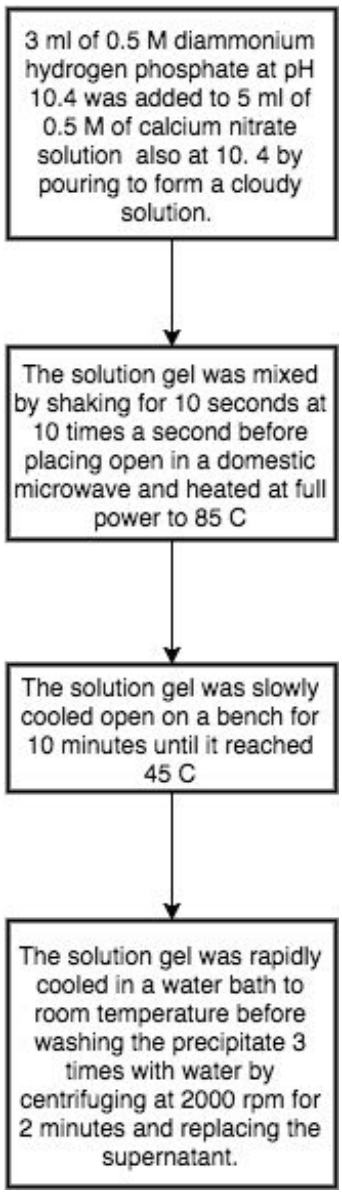


Figure 2.4: The microwave method typically used by this work to make apatite.

3

An investigation into the optimization of the hydrothermal method

Since it was demonstrated in the literature that the hydrothermal method could make highly regular particles that were tailorable [26, 36], it seemed practical to start an investigation of these properties of interest with this approach. It was also noted that in these descriptions, particles (also referred to as crystals in this work) made by this method sometimes demonstrated a microstructure, an internal structure within each particle formed by the assembly of much smaller particles. It was thought that this feature might be useful as it could enhance the toughness of a material assembled from such particles.

As mentioned, the hydrothermal method differs from the other two methods investigated as the gel was heated above boiling point and chelates were added to bind to

and control the particles formed in the gel [33, 34]. Because the solvent was aqueous, the high temperature applied meant that the gel had to be contained within a pressure vessel or a reflux setup.

In this Chapter, aspects of the hydrothermal methods described in the literature [33, 34] were tested to see what changes could be made to the (a) regularity and (b) morphology of the particles produced and the (c) yield of the precipitate.

It was not known at the start of this investigation if the particles with the intended morphology could be made without also disrupting their regularity or if the amount of precipitate that could be produced would be high enough [14]. If it was found to that it was possible to make anisotropic shaped particles in gram-scale amounts, it would make it feasible for their assembly into bulk material with enhanced mechanical properties.

It was thought likely by the Author that a compromise would be made between achieving the desired regularity and the desired yield. It seemed difficult to achieve both of these these goals simultaneously.

3.1 The effect of the gel's ion saturation on the precipitate produced

After testing a citric-hydrothermal method, it was found that the yield was low, typically less than 5%, and the absolute amount produced from an 80 mL volume of gel was a fraction of a gram, which was far less than the gram-size amount per batch that maybe required for a centimetre-sized monolith [9].

Some possible answers to the question of how to increase this low yield could be to (a) prepare larger amounts of gel, (b) increase the initial reagent concentrations added to the gel or (c) implement both approaches. There were, however practical limits to these answers. The maximum volume that could be fitted into the pressure vessel was 300 mL. Most of the precursor chemicals had a limit to the concentration they could

be prepared at, which was usually not more than 1 M, before they became insoluble in water or in the gel mixed with the other reagents.

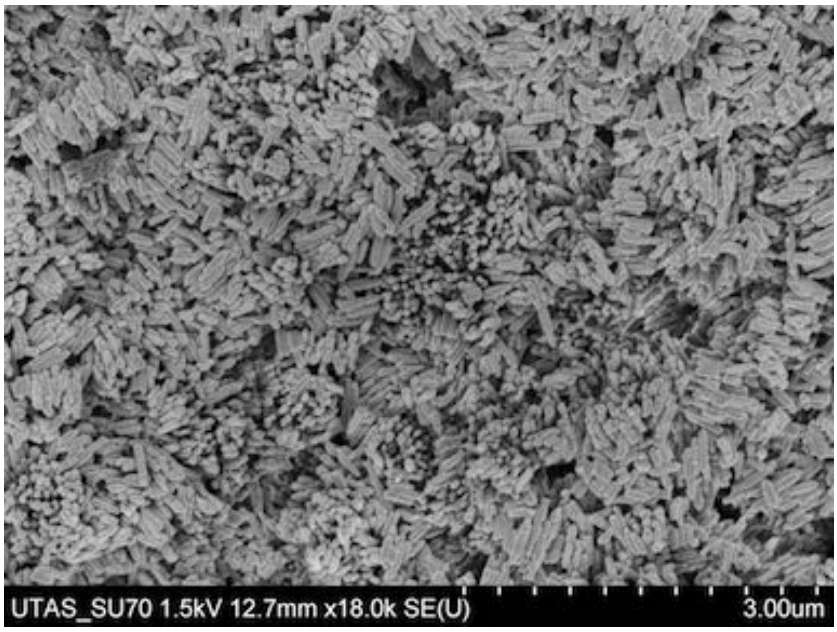
Unfortunately, both of these approaches are work-arounds as they do not address directly the low yield issue and their implementation would lead to the consumption of a large amount of reagents and so were not considered practical to implement.

Nonetheless, it was decided to investigate how concentrated the reagents could be prepared and processed before the reaction or its precipitate was compromised. The reagents were prepared to a 1 M stock concentration. They were then added at a different volume ratios of 5:3:1:2 for calcium, phosphate, fluid and citric acid. The range of the concentrations prepared and tested was 20, 40, 80, 160, 240 and 320 mM.

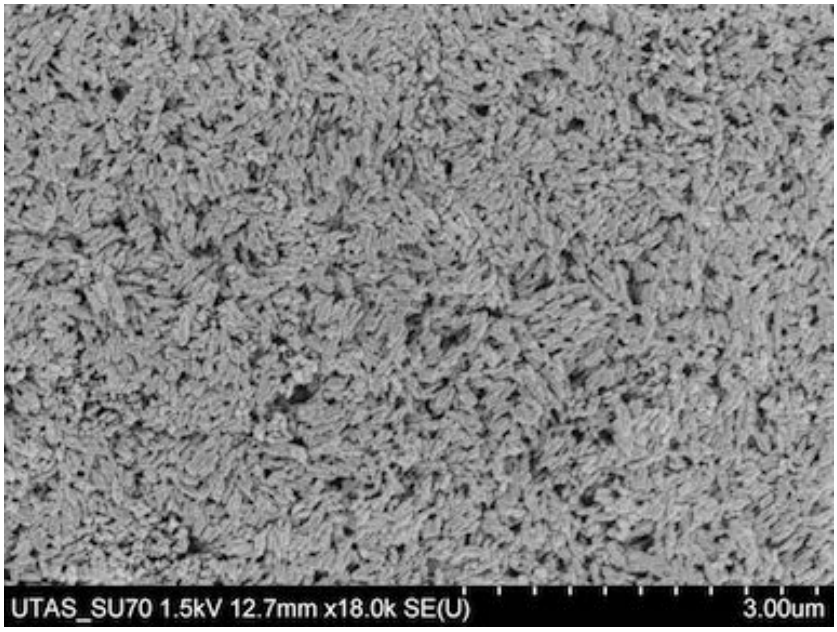
It was found unsurprisingly, that particles were more likely to be regular and crystalline if they were formed from a gel that contained a lower rather than a higher concentration of ions. As the concentration increased from 20 mM, the precipitant produced increased proportionally; a doubling in the concentration caused a doubling in the amount of precipitate.

The tendency of the particles produced to be more regular and crystalline at lower rather than higher concentrations of ions is not surprising. The literature supports the idea that at saturated or supersaturated concentrations of ions in the reaction gel, crystallisation becomes inhibited as its rate is slowed to the point where it failed to go to completion within the time set for the solution gel to incubate [9]. Also it is known that the chelation of particles, by for instance citric acid, may become hindered at higher ions concentrations as their attachment to calcium ions competes with attachment to other ions. These assumptions are further supported by the concentrations that are known to naturally occur in the body when bone or tooth are formed.

This result was consistent until the concentration of the calcium in the gel reached 160 mM, at which point the amount of precipitate did not increase further. Above this concentration, the quality of the precipitate became substantially less regular and thus less crystalline. When the concentration of the solution gel was tested at 240 mM, uniform particles could not be formed and the resulting precipitate appeared amorphous (Fig. 3.1).



(a)



(b)

Figure 3.1: To increase the absolute amount produced by the hydrothermal reaction, the concentration of the reactants was increased. The particles shown as SEM images were produced at a calcium concentration of 240 mM and at a pH of the solution gel set to a pH of 5.2 (Fig. 3.1(a)) and 5.4 (Fig. 3.1(b)).

Possibly the solution gel became oversaturated with ions and the crystal growth became confined. Reports elsewhere used much lower concentrations (a figure of 2 mM was common) and none reported testing at these much higher concentrations [33, 34], and so the methods described elsewhere might not be fully translatable to the one used in this work at those high concentrations. However, it was found that regular particles could be produced up to 160 mM for each reagent, which was about 40 times higher than what was reported elsewhere.

3.2 The effect of adding citric acid and the heating rate of the gel

Most hydrothermal methods in the literature incubate the gel in an oven that was already preheated to a desired temperature [7]. It was thought that it would be simpler (and more interesting as it was rarely reported) if instead the gel was slowly heated from room to a final temperature, for example at a rate of 0.6 °C/min over 4 hours to 160 °C, and then, rather than incubate at that temperature, immediately cool the gel to room temperature [37].

In this test, the solution gel was prepared and then heated slowly until it reached 160 °C after which it was cooled over 10 minutes by placing it in a water bath back to room temperature. It was found that heating a gel, that contained citric acid, at a 0.6 °C/min rate, produced crystals that were more regular than if the gel was placed in an oven already preheated at 160 °C.

It was believed that by slowly heating a solution gel containing citric acid, greater control over heating would provide greater control over its ability to template the forming crystals. Citric acid, a weak organic acid, is known to chelate calcium ions and so can act as a template in the formation of calcium phosphate [30]. The spacing between carboxylate groups is similar in size to the spacing of calcium ions along the c-axis in apatite crystals (Fig. 3.2).

This proved to be the case; it was found that ramping gel temperature at 0.6 °C/min

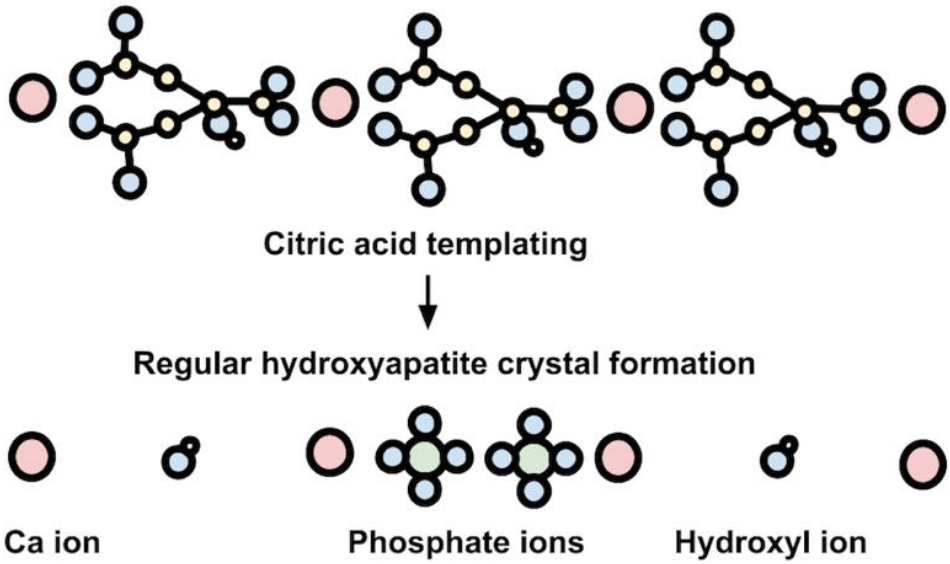


Figure 3.2: Adding citric acid increases of regularity of the crystals since it can act as a template for their formation. A (not to scale) partial model of the crystal lattice of hydroxyapatite is shown templated by citric acid.

produced more regular crystals if the ramping occurred over a range that was at least 100 °C above its starting temperature. As the gel was heated, the pH dropped and, although the ratio of hydrogen ions to hydroxide should remain constant, possibly the rate of calcium ions released and apatite formation became more gradual and thus more controlled [17].

This slow heat treatment of the gel was reported in the literature [21], but in this other report it was done not for the purpose of creating or improving the crystal morphology. It was not tested by the Author, but future work could test this relatively novel approach using a substrate rather than a molecule as a template [1].

3.3 The effect of the addition of EDTA to the gel

Apart from citric acid, ethylenediaminetetraacetic acid (EDTA) is often reported as a calcium ion chelator [32, 34].

In this test, citric acid was replaced with EDTA in the gel and then heated [34, 36]. It was found, surprisingly that unlike these earlier reports elsewhere, the test did not

produce regular crystals, but instead produced crystals that were irregular (Fig. 3.3). Possibly the discrepancy was the much lower concentration of ions used in the gel as described elsewhere [33, 34, 36]. At a high level of ions in the gel, the chelating process much like the precipitation process, can become compromised.

A hydrothermal reaction was performed with the solution gel containing only EDTA at a concentration half what was used for the citric and the same as that for a buffer that was also added. Much like the previous test, the gel was slowly heated at 0.6 °C/min from room temperature until it reached 180 °C, which took just over 4 hours. The buffers used were formic acid at pH 3.8 (Fig. 3.3(a)), propionic acid at pH 4.9 (Fig. 3.3(b)), ammonium acetate at pH 5.0 (Fig. 3.3(c)) and MOPS at pH 7.2 (Fig. 3.3(d)). Irregular rice (Fig. 3.3(a)), string (Fig. 3.3(b)), short rods (Fig. 3.3(c)) and particles with whisker-like profiles (Fig. 3.3(d)) were produced.

Another reason for the less than expected results here was the 4 hour incubation time, which was much shorter than incubation performed and reported elsewhere [31, 33, 34]. Given enough time, the crystallization of ions is known to occur under less than favourable conditions, such as those found in geological contexts, as crystallization can be thought of as the ionic material reaching its most stable and entropic minimal state. In this work, the incubation of the reaction gel was stopped when the precipitate was washed. For the precipitate produced after shorter incubations to reach the same level of crystallization as precipitates incubated for longer, the conditions of the solution gel had to be optimal. However, there is a limit to how short the incubation can be cut, before the precipitate produced is compromised. If the gel was conventionally heated, as reported in this work, this typically occurred when the incubation was cut to less than 4 hours. If a microwave was used to heat the gel, the Author however was unable, to establish the shortest time needed. An estimate of how long it took to heat a given volume of solution gel to 85 °C was used instead; for an 8 ml volume this was found to be 14 seconds of heating with the microwave set to full power.

There are additional details to the description of a conventionally heated precipitate that can be provided. In this work, the gel was not preheated before it was mixed as this was simpler to do. Instead, it was mixed at ambient room temperature and then

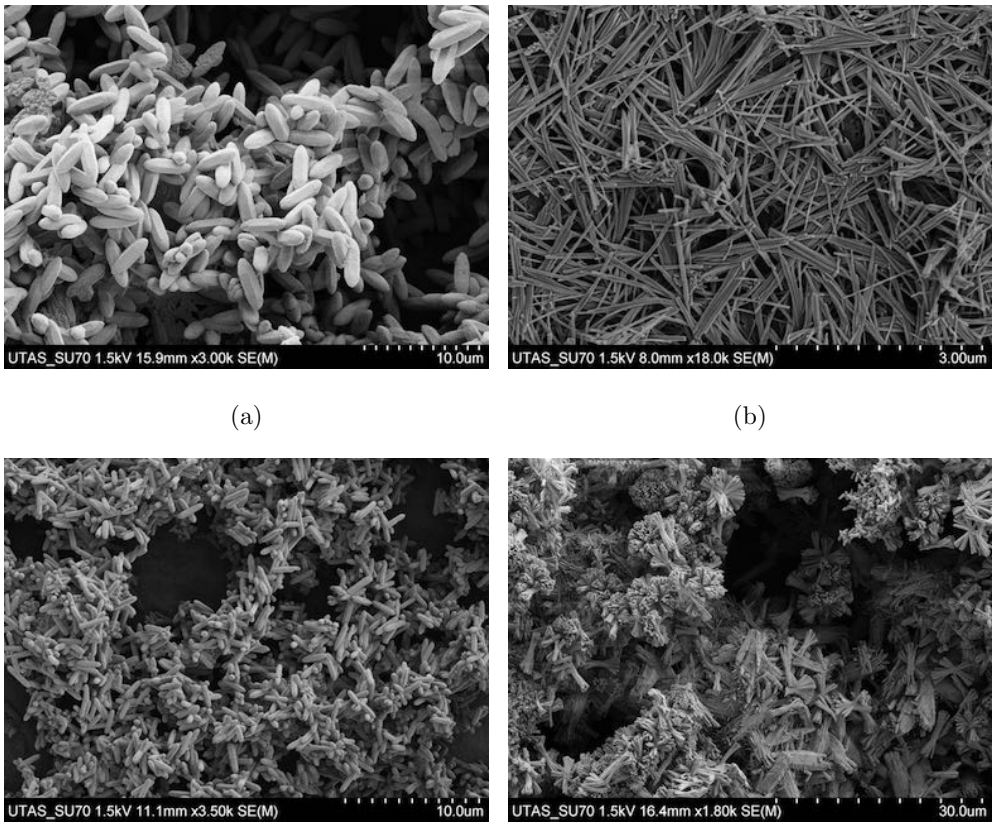


Figure 3.3: Unlike when citric acid was added to the solution gel, the addition of EDTA on its own did not produce regular articles. Particles produced from solutions gels that contain formic acid at pH 3.8 (Fig. 3.3(a)), propionic acid at pH 4.9 (Fig. 3.3(b)), ammonium acetate at pH 5.0 (Fig. 3.3(c)) and MOPS at pH 7.2 (Fig. 3.3(d)) are shown.

placed on a preheated hot plate [31]. It then took the gel in a glass tube 5 minutes, if it was 8 ml in volume, to reach 65 °C, the minimal temperature found in this work that produced crystals of apatite. It then took another 2 minutes for the gel to reach 85 °C, the temperature found to be optimal for making uniform crystals.

3.4 The addition of EDTA and citric acid to the gel

In this test, EDTA was again added to the gel, but this time as a co-chelate with citric acid. The literature suggested it might act as a modifier, adding to the range of shapes reported when citric acid was added to the gel [9, 28].

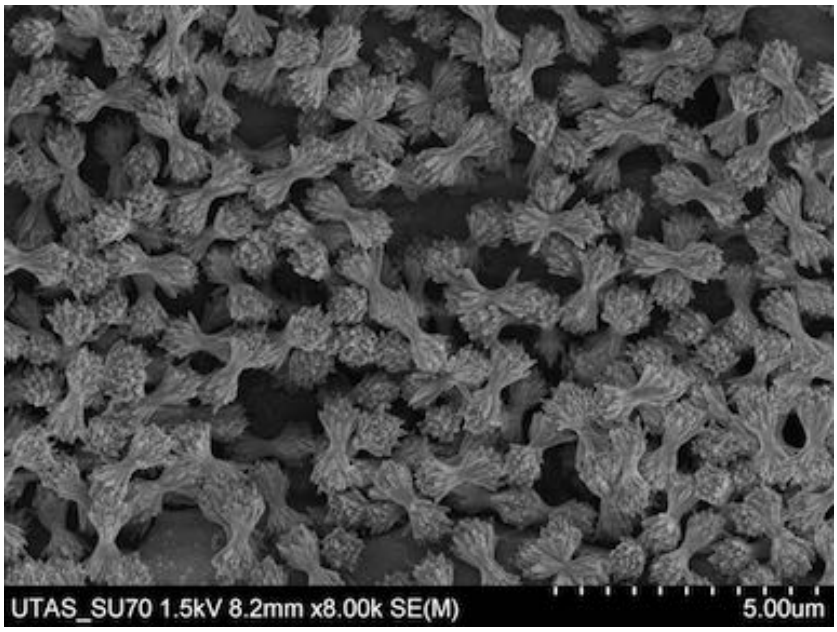
EDTA was added at a volume ratio 1:2:5 EDTA, citric to calcium ions. This ratio of reagents reflects how EDTA was applied as reported in the literature [33, 34] and it also anticipates the mechanisms of each chelate. 2 citrate molecules or 1 EDTA molecule would be expected to bind to a single calcium ion. Various pH levels and reagent concentrations in the gel were also run to test their effect on the precipitate crystals produced.

Unlike the precipitate produced when EDTA was added alone, the particles produced in this test were uniform. Their shapes (as shown in Fig. 3.4) appeared to be modified versions of the shapes made when citric acid was added alone. It appears that EDTA modifies the ends of the particles, making them less blunt and more sharper. These results were in agreement with reports elsewhere [33, 34].

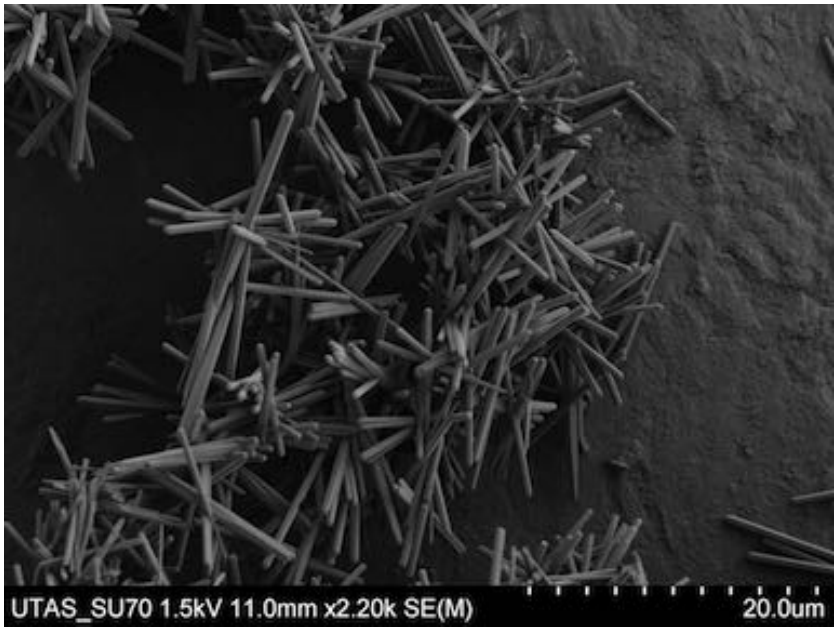
EDTA was added to the gel at half the concentration of the citric acid. The gel contained a BIS buffer set to a pH of 6.7. The crystals were formed after heating to 160 °C and had a whisker-shape (Fig. 3.4(a)). That is, the general form of each particle was rod-shaped, but with each end sprayed out. The underlying rod-shape form can be seen when a HEPES buffer was set to a pH of 8.0, and just EDTA was added and the gel was then heated to 180 °C (Fig. 3.4(b)). This gel formed particles with a rod shape at a high aspect ratio. This result was also reported elsewhere [33, 34].

The precipitate produced was dependent on the pH the solution gel was preset to. It was observed that the particles produced with just citric acid were highly regular between pH 5.7 and 6.7. If EDTA was added to the gel with citric, the range increased to a pH of 4.7 to 6.7. In contrast, if the concentration of the calcium in the gel was increased 10-fold from 20 to 200 mM this range was reduced to pH 5.0 to 6.0.

There were some caveats to these results. Unlike what was reported in the literature, the hydrothermal method used here applied reactants at a much higher concentration; at least 10 to 100 times higher. It then incubated the solution gel for a much shorter time, usually for 4 hours, which was approximately 2 to 4 times shorter than what was typical elsewhere [33, 34]. These changes were put in place to increase the amount of precipitate produced and to shorten the turnaround time for each batch, but these changes made a comparison of the results reported here with those reported elsewhere



(a)



(b)

Figure 3.4: The addition of EDTA with citric acid to the solution gel can provide additional control over the shape of the particles produced. The result of adding of EDTA with citric acid is shown in Fig. 3.4(a) and the result with just EDTA added only is shown in Fig. 3.4(b).

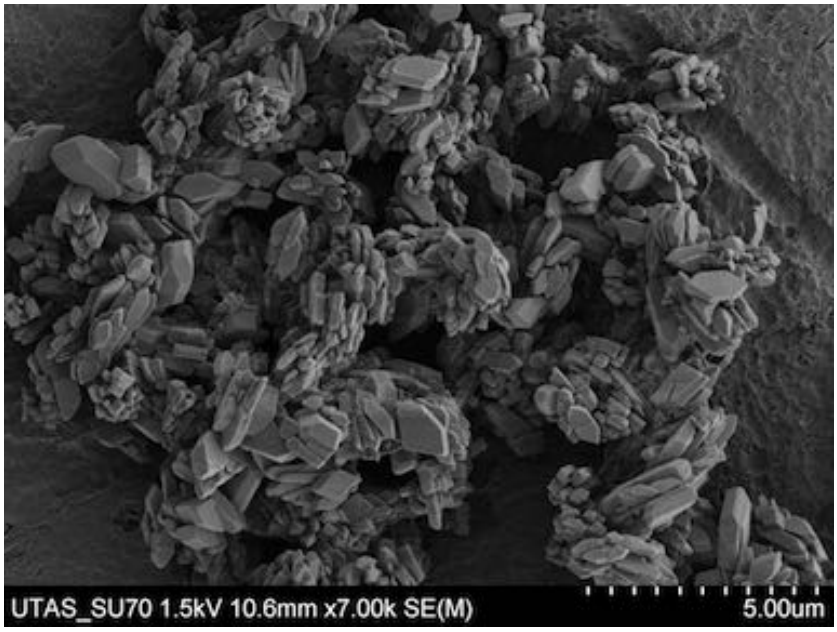
not straightforward.

3.5 Novel solvents and their effect on crystal morphology

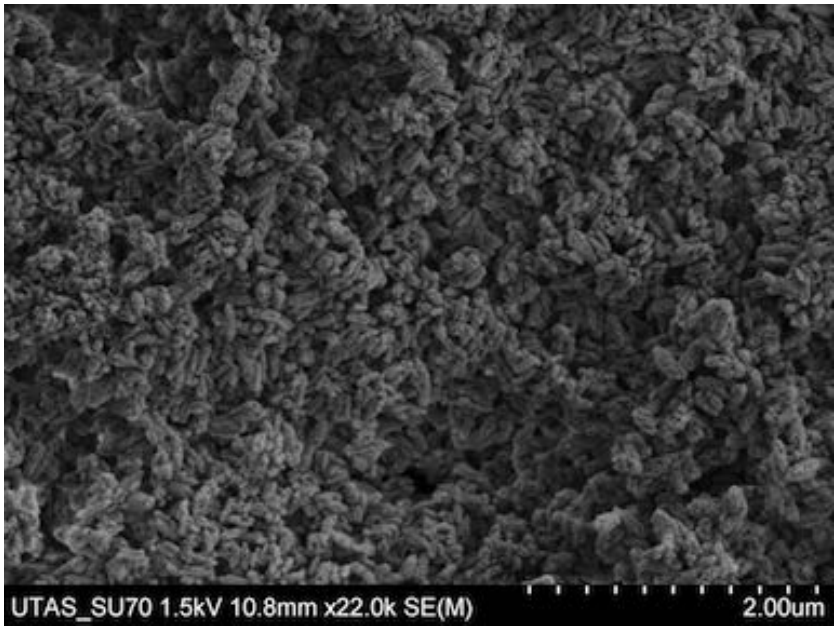
Water is the most common solvent used in the solution gel to make apatite. It is inexpensive and easy to handle, most of the common reagents are highly soluble in water and a precipitate is readily produced [38]. It is rarely reported, but it was considered a useful exercise to test the use of solvents other than water in the solution gel. The solvent potentially could be a major factor in the precipitate produced [7]. Only solvents that could be dissolved (in a 1:1 by volume ratio with water) the reagents used in this work were tested.

In most tests, the inclusion of a solvent other than water was found to disrupt the formation of regular particles and caused the particles to aggregate (Fig. 3.5). The tests were run with a ramped hydrothermal reaction heated for 4 hours to 160 °C. The solution gels tested contained either 50% isopropanol and 50% water with citric acid (Fig. 3.5(a)) or 50% formamide and 50% water with EDTA (Fig. 3.5(b)).

The application of isopropanol or formamide to the synthesis of apatite was found not to improve the quality of the precipitate produced. In contrast to this less than expected result, initial tests with partial aqueous solutions of dioxane proved to be promising, and are reported in Section 4.6. Future work might test the reactants in solvents without any water added, but none were found when testing that could dissolve all the reactants at the high concentrations already tested with water.



(a)



(b)

Figure 3.5: Addition of a solvent other than water typically disrupts the formation of regular crystals. 50% isopropanol and 50% water with citric acid in the gel (Fig. 3.5(a)) or 50% formamide and 50% water with EDTA in the gel (Fig. 3.5(b)) are shown.

3.6 The application of surfactants to the gel to modulate particle morphology

Surfactants, rather than solvents, are more frequently reported in the literature, possibly because their effects were found elsewhere to be positive [39]. In this work, cetrimonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Tween 20 were tested and found, like elsewhere [39], to change the morphology of the particles produced, without disrupting their regularity.

These detergents were added to the calcium solution and dissolved before the phosphate solution was added and the gel processed as before hydrothermally. The shapes of the crystals produced were similar to those made when the pH of the gel was changed (Fig. 3.6). With the addition of a surfactant, the crystals changed from a rod to a whisker-like shape or the reverse from a whisker-like to a rod.

In this test, a hydrothermal gel with a BIS buffer at pH 6.7 was heated for 4 hours to 160 °C. As shown in Fig. 3.6(a), a control was run without a surfactant and whisker-shaped particles were produced. When cetrimonium bromide (CTAB) was added, the particles became more rod-shaped (Fig. 3.6(b)). Again when sodium dodecyl sulfate (SDS) was added, the particles became more whisker-like, as shown in Fig. 3.6(c). When Tween 20 was added, as can be seen in Fig. 3.6(d), the particles became more pronounced in their whisker-shape. These particular results were not reported elsewhere.

Possibly, the change that occurs when a surfactant was added to the gel is similar in effect to the change that can occur when the pH of the gel is modified; the surfactant modifies the surface of the growing crystal, possibly decreasing or increasing the strength of its dipole moment [30]. As these tests were run, it was found that it was difficult to apply surfactants at concentrations higher than 20 to 40 mM as their viscosity increased and it became to difficult to add them to the gel.

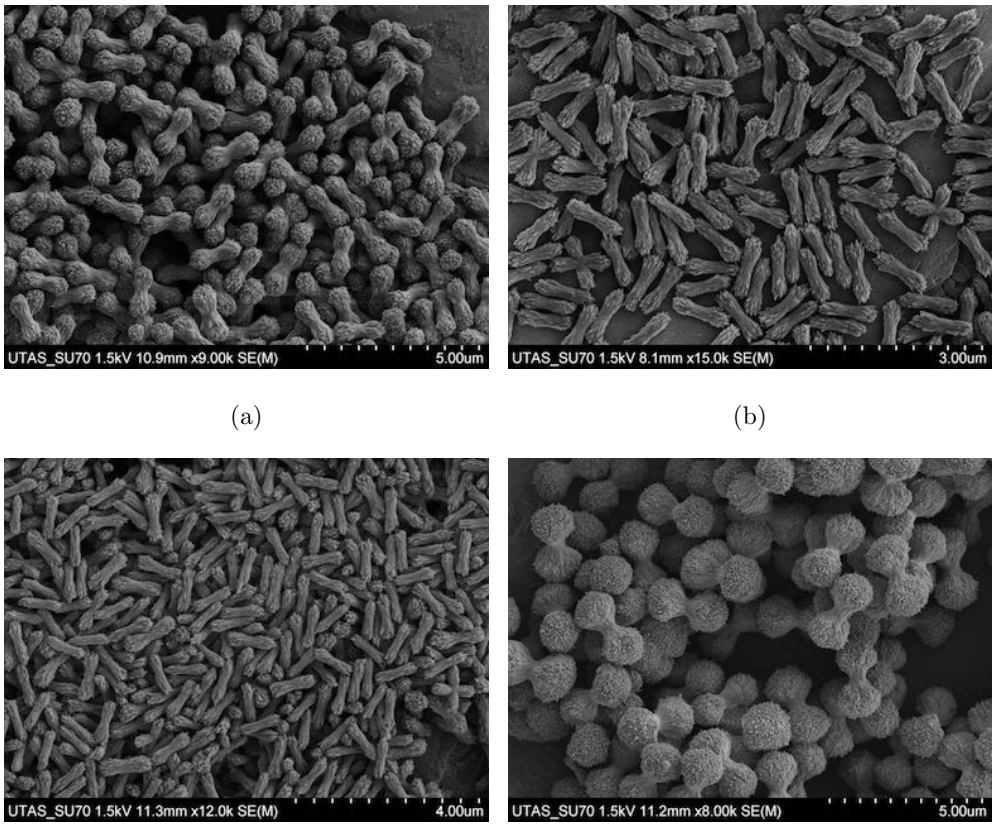


Figure 3.6: Surfactants were used to control and modify the shape of the particles produced. A control was run without a surfactant and whisker-shaped particles were produced (Fig. 3.6(a)). The addition of cetrimonium bromide (CTAB) produced rod-shaped particles (Fig. 3.6(b)). A similar outcome occurred when sodium dodecyl sulfate (SDS) was added (Fig. 3.6(c)). When Tween 20 was added the particles had a pronounced whisker-shape (Fig. 3.6(d)).

3.7 The fabrication of cube-shaped particles after the addition of magnesium and oxalic acid

Adding another metal ion to the gel to fully or partially replace the Ca^{2+}, was thought to be a useful test for the possible generation of new shapes. Tests were run to see if it was possible to generate novel shapes. New shapes might provide new opportunities for their assembly into a bulk material.

Doping the gel with metal was frequently reported in the literature and was typically

performed to add to or change, usually chemically, a property of the precipitate [15]. Rarely is the possible effect this might have on the shape of the crystals produced the focus of a test. To test the role of metal doping, magnesium (Mg) or zinc (Zn) ions were added to the solution gel at the same ratio as the fluoride ions when they were added; 5 calcium ions to 1 metal ion.

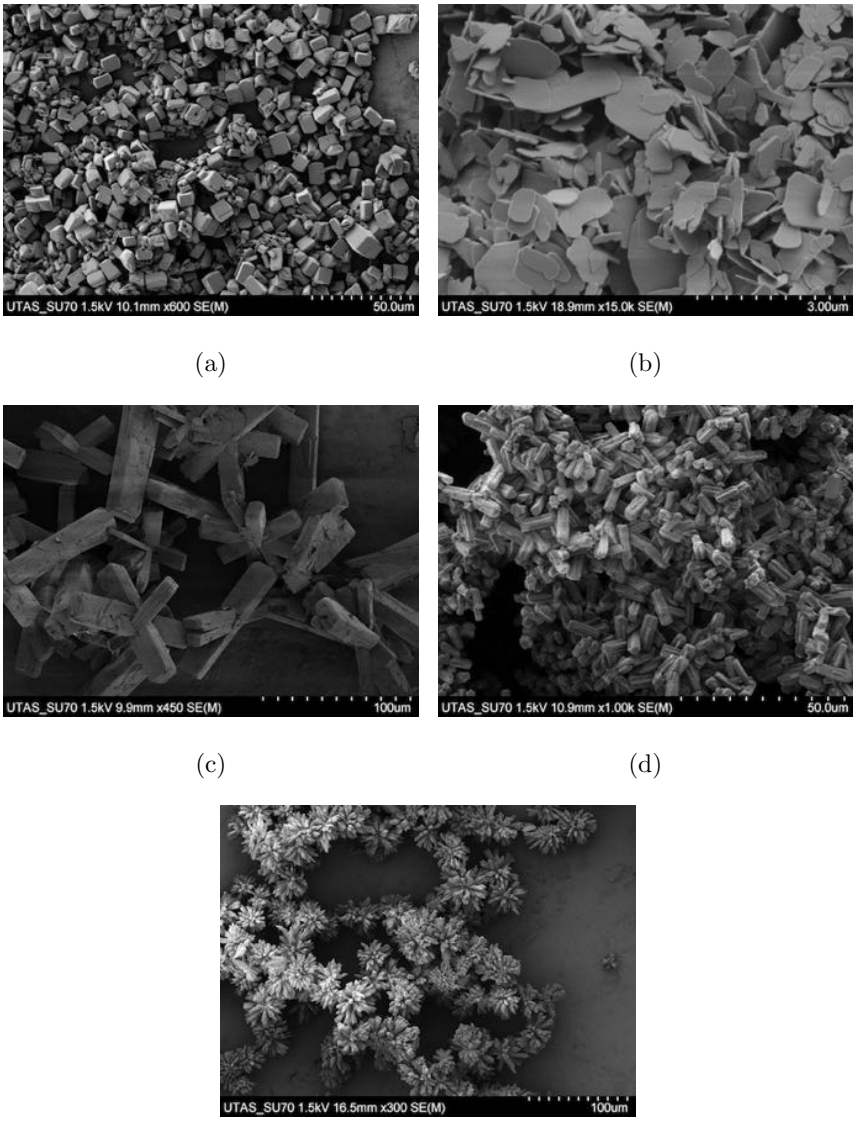
Fig. 3.7(a) lists images of the cubic blocks produced after oxalic acid and Mg were added to a gel containing 50% hexane that was buffered with ammonium acetate set to pH 4.8. Fig. 3.7(b) illustrates that Zn added to the gel produced flat fakes. Fig. 3.7(c) shows that oxalic acid and Zn added to a gel buffered in propanoic acid at pH 4.9 produced intersecting wood-like particles.

Adding Zn and EDTA with the citric acid and buffering in BIS at pH 6.7 produced brick-like shapes as shown in Fig. 3.7(d). Fig. 3.7(e) shows that flower-shaped particles were produced by again adding Zn and EDTA, but this time with a HEPES buffer set at a pH of 7.7. It appeared as if doping with either Zn or Mg produced novel shaped crystals, but crystals which were irregular and had a low crystallinity.

These results were in agreement with the literature [15]. Tests (not shown) where the calcium ion was fully replaced with a metal ion were did not meet expectations as they were far less regular (data not shown). Since the particles that resulted from partial or full metal doping were irregular and amorphous, this work did not do further tests with metal doping.

3.8 The production of regular morphology hydroxyapatite particles

A theme of this work was simplicity; to reduce the method to its most minimal and shortest form without compromising the particles produced. It was thought important to develop methods that were minimum in their complexity as these would make it more straightforward to produce large amounts of the desired precipitate. One approach taken in this work was to run a conventional method, but



(a) (b) (c) (d) (e)

Figure 3.7: The addition of metal ions to the solution gel formed particles with new shapes, but also with less crystallinity. Fig. 3.7(a) shows the result when oxalic acid and Mg were added. Fig. 3.7(b) shows the result when Zn was added. Fig. 3.7(c) the result when oxalic acid and Zn was added. Adding Zn and EDTA is shown in Fig. 3.7(d). Fig. 3.7(e) shows the result when Zn and EDTA were added, but with a different buffer.

with an aspect or step not included to test, with this omission, if it could still make apatite and still make regular particles.

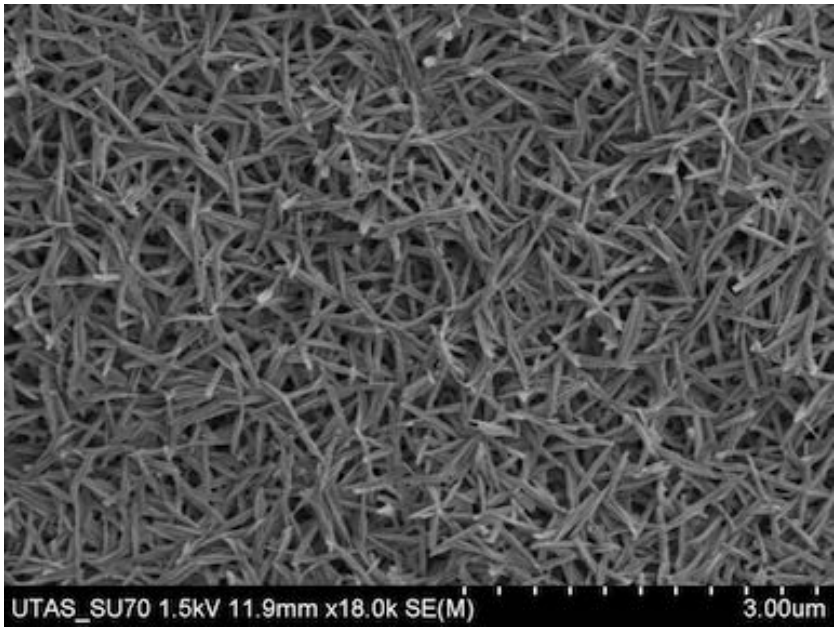
To test if regular particles of hydroxyapatite could be produced, the hydrothermal method was repeated with citric acid, but without fluoride added to the gel (Fig. 3.8). Unexpectedly, since it was not explicitly reported in the literature, it was found that it was possible to make (slightly less) regular particles of hydroxyapatite.

Fluoride was previously found in this work to increase the crystallisation of the particles. Without it, as shown in Fig. 3.8, the particles were less regular and were also less solid or thick, but these were still considered by the Author acceptable for its intended later purpose. It was found that, unlike fluorapatite which had an optimal pH of 5.4, the optimal pH for hydroxyapatite was higher at 6.0. As the concentration of calcium in the gel tested increased from 20 mM to 160, this value for hydroxyapatite increased further to pH 6.6.

For this test, the solution gel containing EDTA (Fig. 3.8(a)) or citric acid (Fig. 3.8(b)) with the pH tested at different levels and the gel without the addition of fluoride, were prepared. The gels were slowly heated for 4 hours to 180 °C and then cooled as before. A precipitate was produced, and like the previous tests with EDTA was irregular, but unlike fluorapatite, this time was a bit larger in size. The citric acid precipitate was again regular, but this time smaller in size and less crystalline than particles produced when the gel contained fluoride. The low yield produced by this hydroxyapatite method was similar to the low yield produced when fluorapatite was made. The yield data is reported in Chapter 6.

In another test (data not shown), instead of citric acid, EDTA was added and again without fluoride in the gel. The particles became irregular, but were also uniquely shaped. It might be that the results seen here could be improved by testing in future work the gel at a lower concentration of reagents.

(a)



(b)

Figure 3.8: It was possible to make regular particles of apatite without fluoride. Fig. 3.8(a) shows particles made with EDTA and without fluoride in the gel and Fig. 3.8(b) shows particles made with citric acid and without fluoride in the gel.

3.9 The effect of the pH of the gel on the shape of hydroxyapatite particles

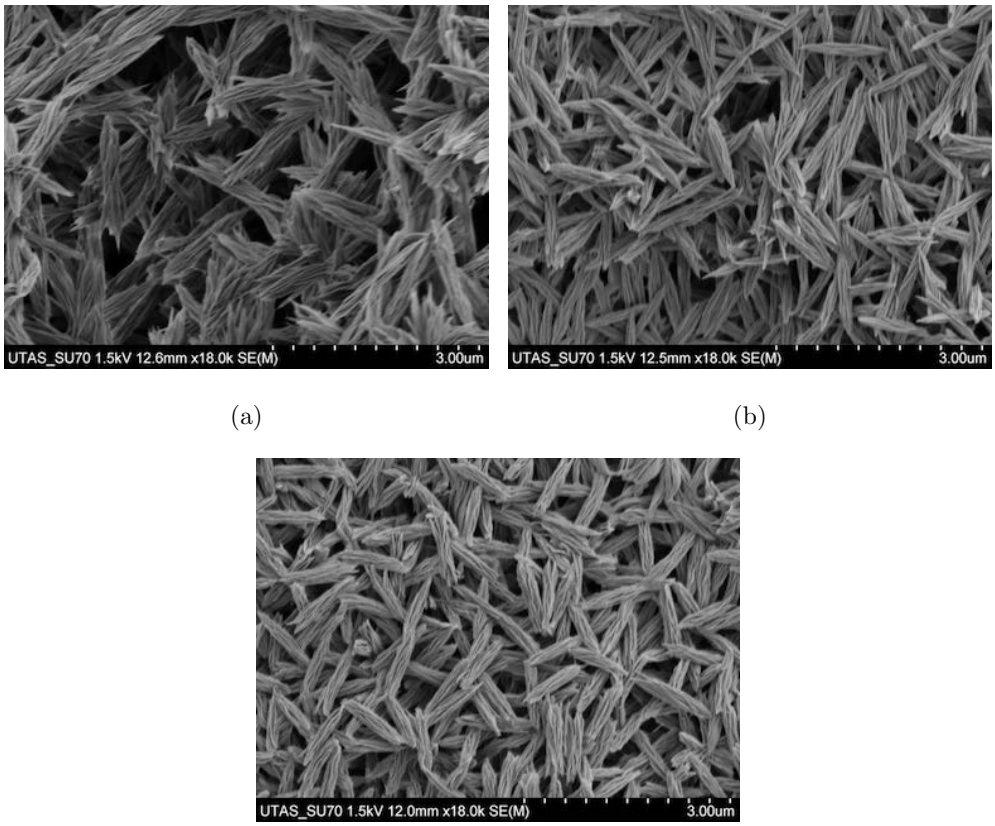
It is shown in Section 3.8 that regular particles of hydroxyapatite could be produced without the addition of fluoride. To test if the regular shape of these hydroxyapatite particles was as sensitive to the pH level in the gel as fluorapatite, gels set to different pH levels were tested.

Little change in the shape of the particles produced was seen at various gel pH (Fig. 3.9). This was unexpected and contrary to the results found when fluorapatite was made. The apatite crystals had the same appearance when formed from solution gel set to various pH tested from 5.4 to 6.4. The rod-shaped particles made of hydroxyapatite appeared to be less solid than those made of fluorapatite reported in this work.

There was some differences seen with changes to the pH. It was observed that at a lower pH the particles were larger, as shown in Fig. 3.9(a). This was not reported before. It is unknown why no change was seen at the various pH tested in the gel, but possibly a more comprehensive test across a larger range of pH might provide greater insight.

3.10 The control of fluorapatite particle morphology by adjustment of the solution gel pH

Fluorapatite was characterized by XRD and Zeta and the results shown in Fig. 3.10 These results were consistent for all methods of apatite synthesis tested in this work if the solution gel was doped with fluorapatite. The two factors that appeared to control the morphology of fluorapatite particles were the pH and the temperature conditions in the gel [17]. These conditions also appeared to be interrelated, each having an effect on the other. Two experiments to test this were run. In one test, the pH of the gel was set between 3.6 and 7.6. In the other test, particles produced from a gel set at both a low pH and incubation



(a) (b)

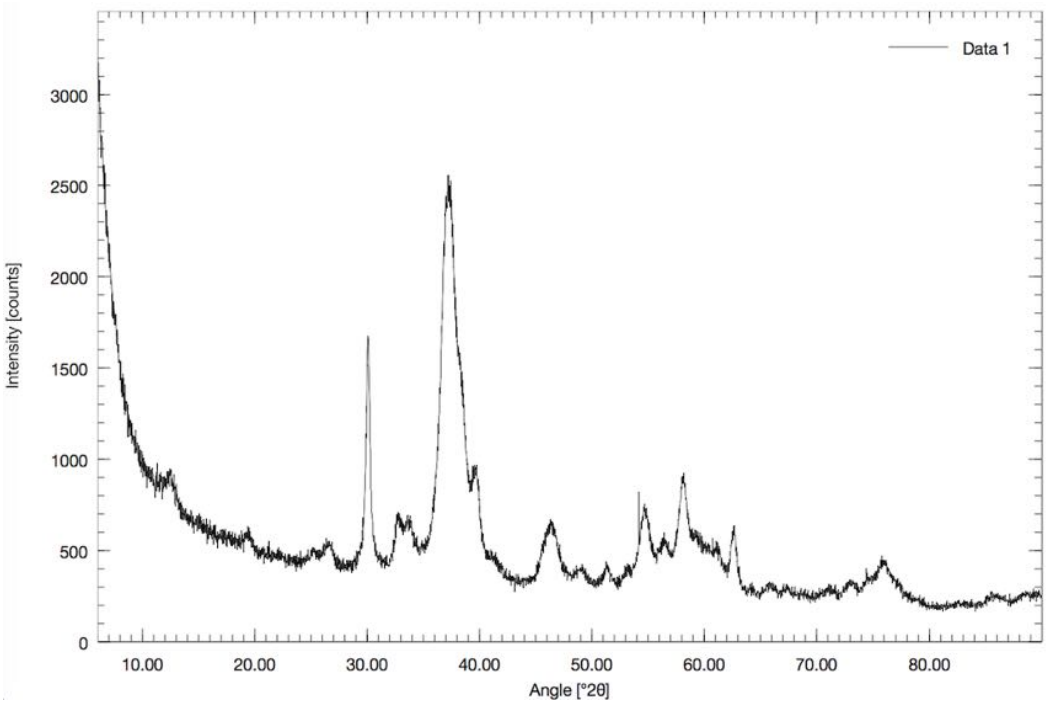
(c)

Figure 3.9: The pH of the solution gel has much less of an effect on the shape of particles of hydroxyapatite produced than it does on particles of fluorapatite. At a lower pH, as seen in Fig. 3.9(a), the particles were larger. At a high pH, as seen in Fig. 3.9(b) and in Fig. 3.9(c), they were smaller and more solid. By observing the SEM images, some of the particles appear to be less solid i.e. to have a porosity, an internal fugitive structure to them then other more solid less porous particles.

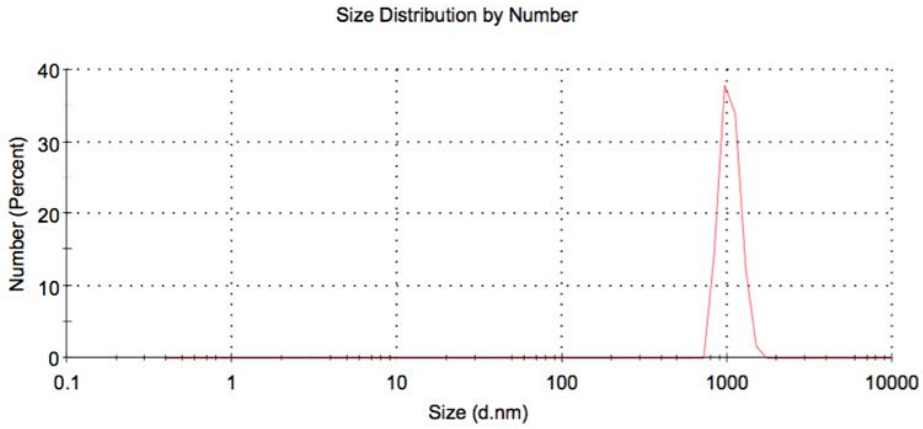
temperature was compared to one set at both a high pH and incubation temperature.

It was observed in Fig. 3.11, that as the pH of the gel tested rose from low to high, the shape of the fluorapatite particles also changed. It was also found that at a low or high pH, the synthesis of the particles was compromised and an amorphous precipitate was produced. At a mid-pH, particles were at their largest and, as the pH rose in the gel from low to high, the particles produced became less rod-like and more whisker-like [17].

The fluorapatite particles produced were irregular at pH 3.6 (Fig. 3.11(a)) and 7.2 (Fig. 3.11(j)) and 7.6 (Fig. 3.11(k)). They were regular at pH 4.0 (Fig. 3.11(b))



(a)



(b)

Figure 3.10: Fig. 3.10(a) XDR analysis of precipitate indicated fluorapatite. Fig. 3.10(b) The particle size distribution indicated that the particles in solution were about 1 micron in size.

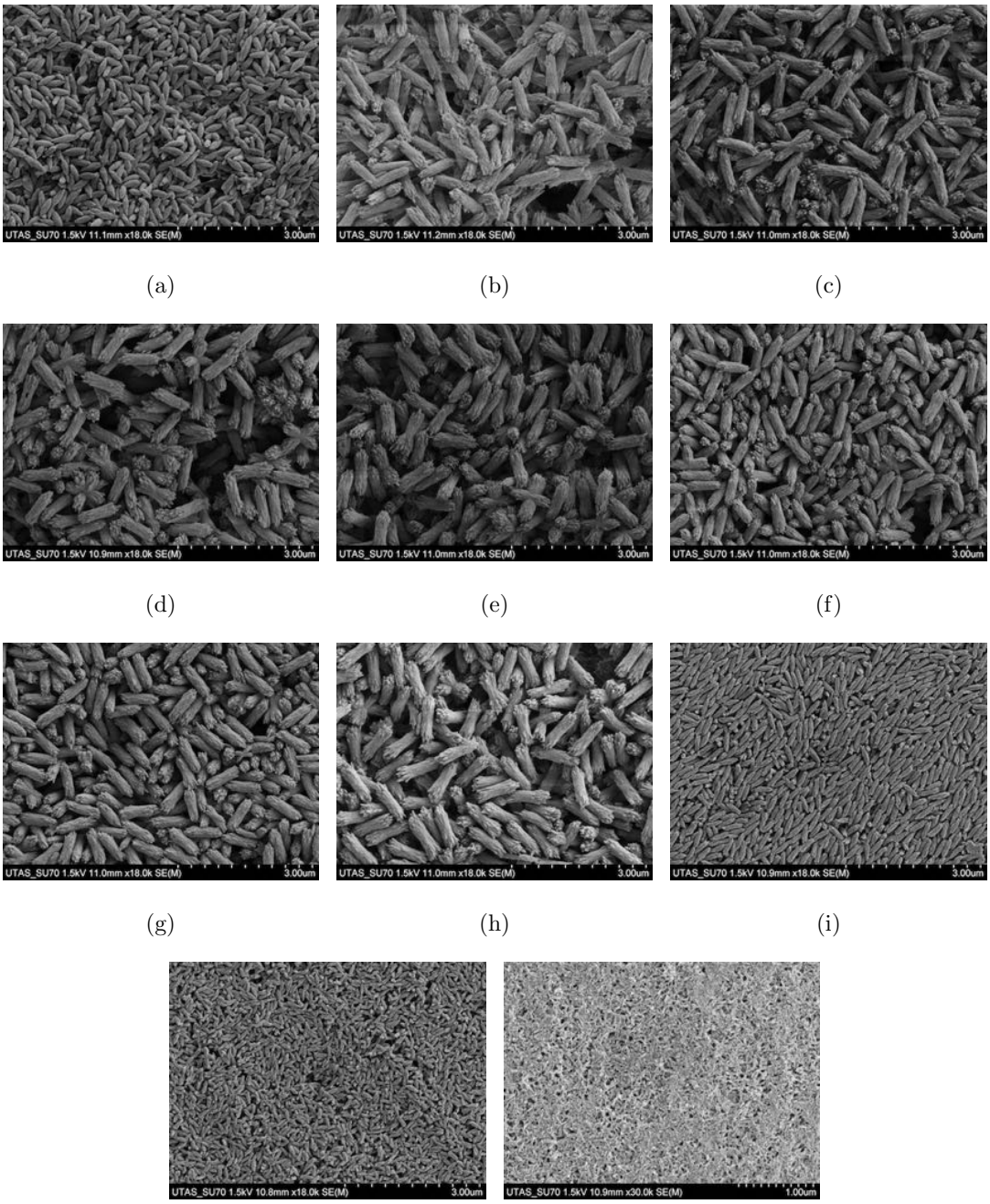


Figure 3.11: The pH of the solution gel is a major influence on the shape of fluorapatite particles. Irregular particles were produced at a pH of 3.6 (Fig. 3.11(a)), 7.2 (Fig. 3.11(j)) and at 7.6 (Fig. 3.11(k)). They were regular between a pH of 4.0 (Fig. 3.11(b)) to 6.8 (Fig. 3.11(i)).

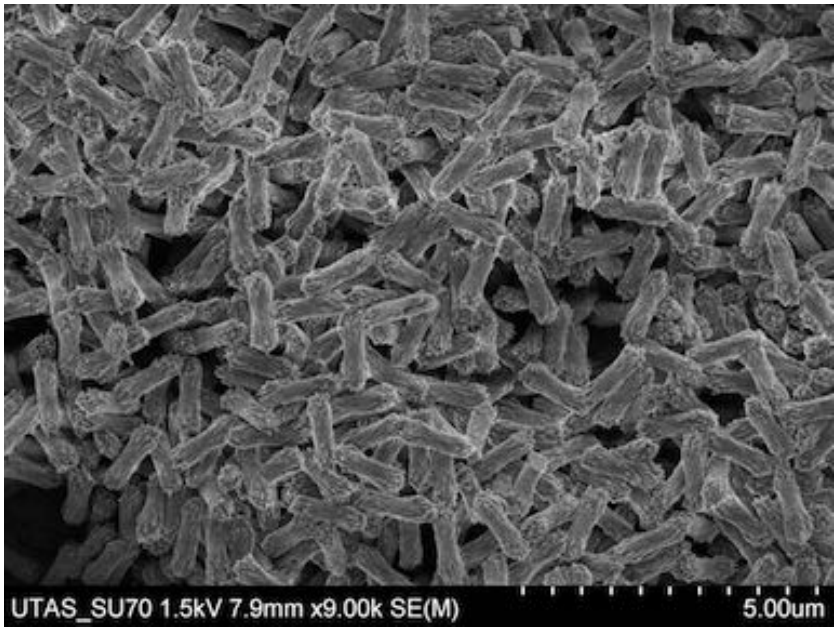
to 6.8 (Fig. 3.11(i)). They had an almond shape at pH 3.6 (Fig. 3.11(a)), and 7.2 (Fig. 3.11(k)), and a rod shape at pH 4.0 (Fig. 3.11(g)) to pH 6.0 (Fig. 3.11(b)). They had a whisker shape at pH 6.4 (Fig. 3.11(h)). They were largest at pH 4.8 (Fig. 3.11(d)) and 5.2 (Fig. 3.11(e)). They were smallest at pH 3.6 (Fig. 3.11(a)), and pH 6.8 (Fig. 3.11(i)) to 7.6 (Fig. 3.11(k)).

In a second test, shown in Fig. 3.12, fluorapatite made at a low temperatures and pH, were found to be similar in shape to particles made at a high pH and temperature. Specifically, particles made at 150 °C and a pH of 5.2 matched those made at 180 °C and a pH of 7.2. Similarly, particles made at 160 °C and a pH of 6.0 matched those made at a 180 °C and a pH of 7.7. The pH and temperature of the gel appear to be interrelated. It is commonly known that as the temperature of a gel rises, its measured pH drops.

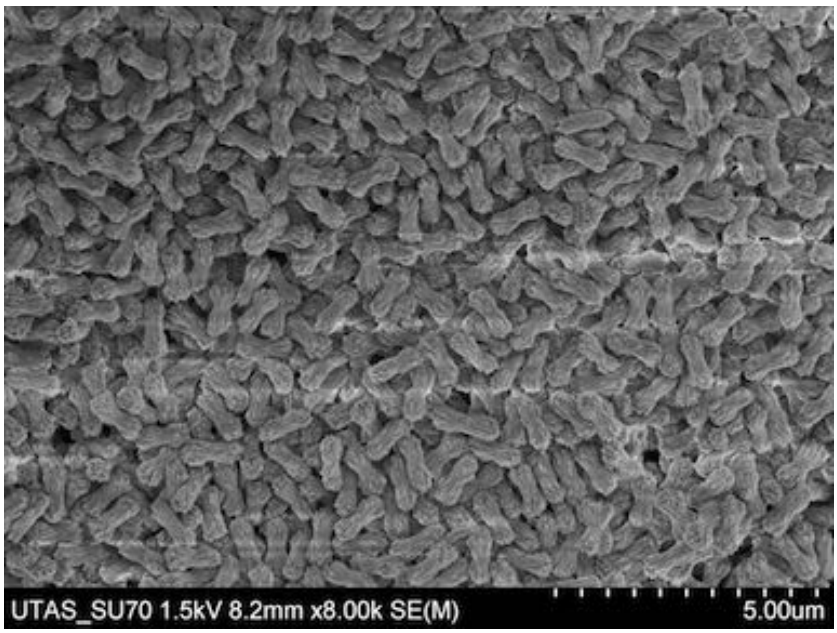
For further information on the phenomena of the pH of a gel increasing as it is heated, the Author refers the reader to the following literature [31, 33, 34]. This general physical phenomena has also been reported in the literature specifically in relationship to the synthesis of calcium phosphates.

These results suggest that it was possible, when making fluorapatite, to control the size, shape and regularity of particles by changing the solution gel pH. Similar results were reported elsewhere [37, 40], but this work is the first systematic test of the effect of different gel pH and incubation temperature on the shape of the crystals of fluorapatite produced.

A change in the shape of the particle as the pH level was increased might be explained, and is illustrated in Fig. 3.13, by an increase in the dipole moment that surrounds each particles as it grows [30]. The presence of an electric dipole field created as the calcium cations coalesce with the phosphate anions might align the citric ions parallel to the c-axis of a forming fluorapatite crystals.



(a)



(b)

Figure 3.12: The pH and temperature of the solution gel were interrelated in their determination of the shape of the particles produced. As shown in Fig. 3.12(a), whisker shaped particles were produced at a pH of 5.2 and 50 °C. As shown in Fig. 3.12(b), similar shaped particles were produced at a higher pH of 7.2 and an incubation temperature of 180 °C.

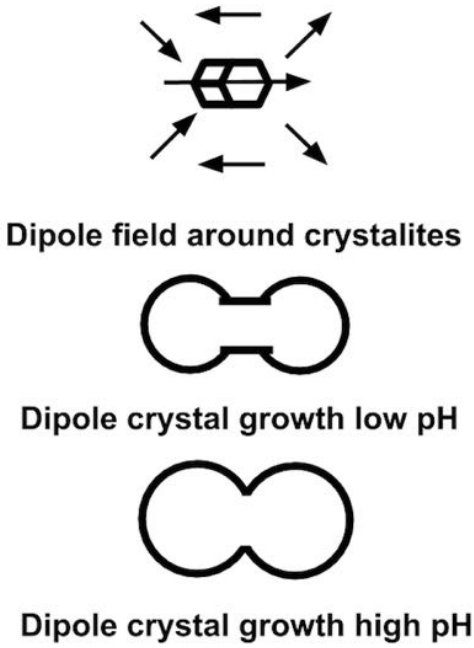


Figure 3.13: A dipole moment might provide an explanation for the shapes observed when citric acid was added to the gel. As shown from top to bottom in this cartoon of a particle forming in the presence of an electric dipole field created by the apatite ions as they coalesce, the citric ions align parallel to this field and direct the particles to form a dumbbell or whisker shape. This effect was enhanced at a higher pH.

3.11 Increasing crystallinity by increasing the reaction temperature

An observation drawn from these results was that heating of the gel to a high enough temperature was needed to make large crystal particles [31]. To confirm if this was true, the solution gel was not heated, but left on the bench. The particles that were produced (not shown) were small, spherical and irregular in shape, even after two days of incubation (data not shown).

Another test, not done in the work because of time constraints, would be to heat the gel to the same final maximum temperature, but incubate the gel for different lengths of time. Since temperature and time were interrelated, it would be expected that a long incubation at low temperature would produce the same level of crystallinity as a short incubation at a high temperature.

3.12 The relationship between pH and temperature of the gel and particle morphology

The terms regular and irregular particles were already defined in this work. In some examples, however well individual particles were themselves regular, but were also conjoined to other particles: their forms overlapping and intersecting with each other as seen in Fig. 3.3 and were thus not defined as regular. In another example, shown in Fig. 3.3(d), the particles were two types, each regular in themselves, but were found mixed together in the same precipitate and so the precipitate was defined as irregular. In another example, seen in Fig. 4.2, although the particles were regular in their overall size and shape, the ends of each particle were not consistent as their profiles differed, and thus again were considered irregular.

To conclude, as described in Section 3.1, we used two approaches to increase the absolute amount of precipitants produced hydrothermally. One answer was to increase the concentration of the solids, the other to shorten the method to 4 hours so that it could be repeated more often in a single session.

The concentration of the precursor reagents was varied. Crystallisation of the precipitate was found to be dependent on the concentration of the solution gel. If the concentration of the chemicals to be mixed is greater than 0.5 M, two phases are developed in the precipitate, one crystalline and the other amorphous.

Both of these approaches, however did not address an underlying issue that the low yield obtained was caused by the addition of a chelate, such as citric acid, to the gel. The yield data is reported in Section 6.7.

In contrast to incubating the gel at a fixed temperature, as reported in Section 3.2, a test was run where the temperature of the gel was increased slowly from room temperature to a final temperature. This appeared to increase the regularity of crystals, but only if the rise in temperature was larger than 100 °C.

It was observed in Section 3.3 that the addition of EDTA to the solution gel did not increase the uniformity of the particles, a finding at odds with the literature [33, 34]. Possibly the templating and regular particles expected were confounded by the high

concentration of reactants tested in the gel and the short incubation time that was applied.

Apart from surfactants such as SDS or Tween 20 (Section 3.6), chelates such citric or oxalic acid (Section 4.5) and solvents such as water or dioxane (Section 3.5), most reagents added to the solution gel disrupted crystallization.

It was described in Section 3.8 that it was possible to make regular particles hydrothermally without the addition of fluoride. The formation of uniform hydroxyapatite was unexpected and provides support for the approach taken in this work to run fabrication protocols that omit aspects or parts reported elsewhere in an attempt to further simplify these protocols.

It was observed in Section 3.9 and in Section 3.10 that the pH and temperature of the solution gel were interrelated when it came to the shape of particles produced; a change in one was equivalent to a change in the other. **It was also observed that fluorapatite particles made from a gel set to a low pH and temperature matched those made in a gel set at a high pH and temperature.**

An implication of the outcomes from this Chapter is that some aspects of procedures reported in the literature may not be necessary in the fabrication of regular particles. In Section 3.8 it was also demonstrated that regular particles of fluorapatite and hydroxyapatite could be made without citric acid, as far as the Author was aware, a finding not reported elsewhere.

Next in Chapter 4, a further extension of the hydrothermal method optimized in this Chapter will be reported and novel ways to produce particles with novel shapes will be introduced.

4

The development of a novel hydrothermal method for making apatite

In Chapter 3 a description was given of an extension to the conventional hydrothermal method reported elsewhere. It was thought that a further development of this method might be useful and as well provide an opportunity for new insights and outcomes. For instance, it is not known if the fabrication of other particle shapes are possible or if the hydrothermal method can be repeated without the need for high pressure or temperature conditions.

4.1 The addition of amino acids to the gel to modulate the shape of the apatite

The addition of amino acids to the gel was found in the literature to modify the shape of apatite particles produced [7]. To repeat and extend this, amino acids were selected that had sufficient solubility (so that the amino could be added to the desired concentrations without precipitation) in water and were added at the same concentrations as the citric acid when it was added to the gel.

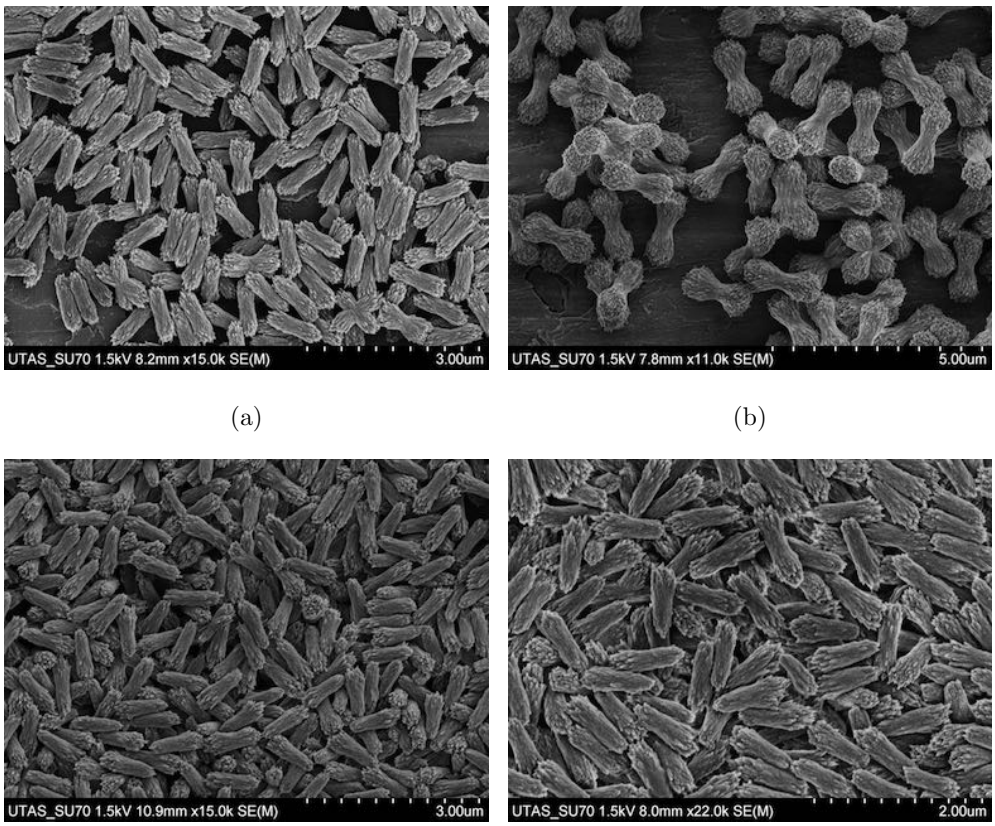


FIGURE 4.1: Some readily soluble amino acids added to the solution gel modified the shape of the particles produced. When compared to a control, cysteine had little effect except to increase the regularity of the rod-shaped particles produced (as shown in Fig. 4.1(a)). Glycine modified the rod-shaped particles to a whisker shape (Fig. 4.1(b)). Arginine (Fig. 4.1(c)) and lysine (Fig. 4.1(d)) produced particles that were asymmetrical, thinner at one end, and wider at the other.

Additional additives in the solution gel can further alter the particle shape already

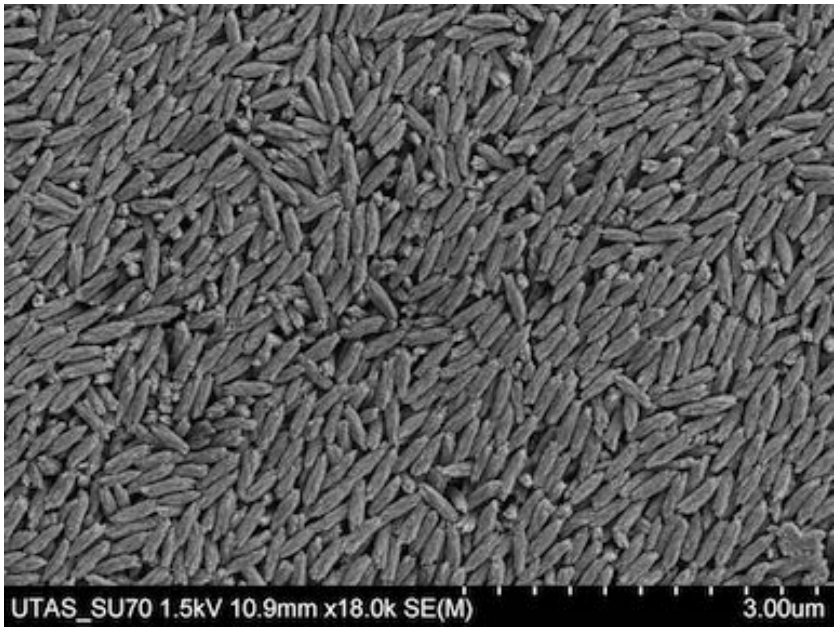
modified by citric acid. Amino acids contain chelating properties and are known to interact with and be central to apatite bone formation [7]. We believed that the addition of amino acids would modify the crystal formation of the apatite produced. In Fig. 4.1(a), results are shown for different soluble amino acids added to the reaction gel and then incubated for 4 hours. Cysteine had little effect on the rod shaped particles except to increase their regularity. (Fig. 4.1(b)). Glycine produced whisker shapes particles. (Fig. 4.1(c)). Arginine and lysine produced rod-shaped particles thin at one end and wide at the other (Fig. 4.1(d)).

The tapering effect of some amino acids on the particles was found elsewhere in the literature [41], but was not reported with the specific amino acids used in this report. There were some caveats to the tests performed here. The tests were performed with a buffer also added to the gel, which in some instances and also shown in this report, can affect the shape of the particles. Furthermore, the testing of these acids was limited. For instance they were tested at a single concentration and at the standard pH. Further testing might provide more insights.

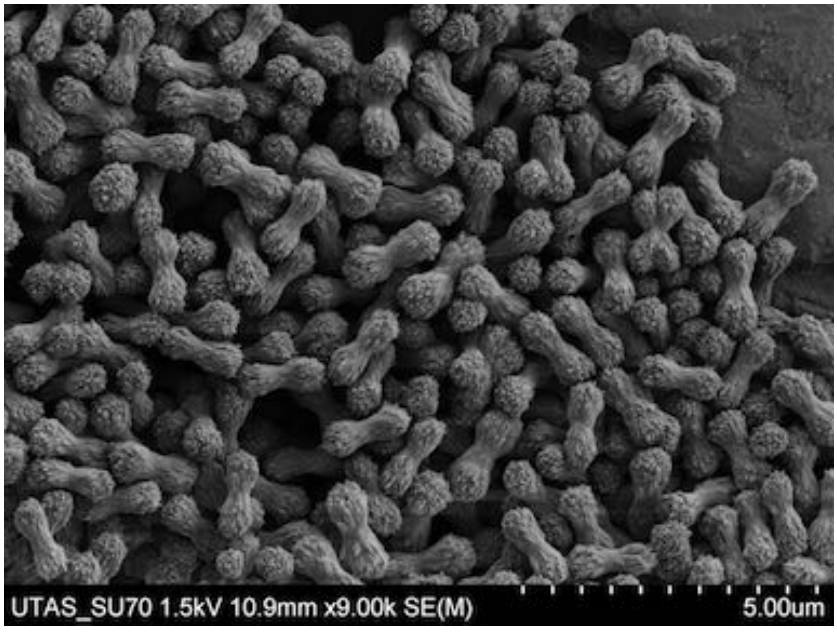
4.2 The use of buffers in the gel to increase the regularity of the particles produced

There are a few reports elsewhere where a buffer was added to the solution gel [42], and no reports, as far as the Author was aware, where it was explicitly used to modify particle morphology. A buffer added to the gel would not be expected to reduce the drop in pH as its temperature was increased since the buffer pKa would also drop, but it was reasonable to assume that it would reduce the pH volatility of the gel as it was first prepared and then heated.

Particle shape was shown in this report to be affected by the pH of the solution gel. Buffering the gel might reduce the sensitivity of this pH to changes brought on by changes in the temperature of incubation. In Fig. 4.2 we tested if adding a buffer increased particle regularity. A hydrothermal reaction was run for 4 hours. The



(a)



(b)

FIGURE 4.2: The addition of a buffer to the solution gel increased the regularity of the particles produced. The results of a control are shown in Fig. 4.2(a). The particles produced with a BIS buffer set to a pH of 6.7 and added to the solution gel, was shown in Fig. 4.2(b).

solution gel without the buffer (Fig. 4.2(a)) was smaller and less regular than the one (Fig. 4.2(b)) with a buffer added.

It is unknown if the buffer itself or its affect on the solution gel's pH helped to increase particle regularity. There were, however some disadvantages to buffering. As an additional additive, it did increase the total concentration of ions in the gel and, in some tests, apart from its buffering effect, it had a modifying effect on particle shape. There was a limit to how much could be added before the reaction and precipitate became compromised. As a result, if a buffer is added, the maximum concentration of the reagents in the gel was reduced to accommodate this new addition.

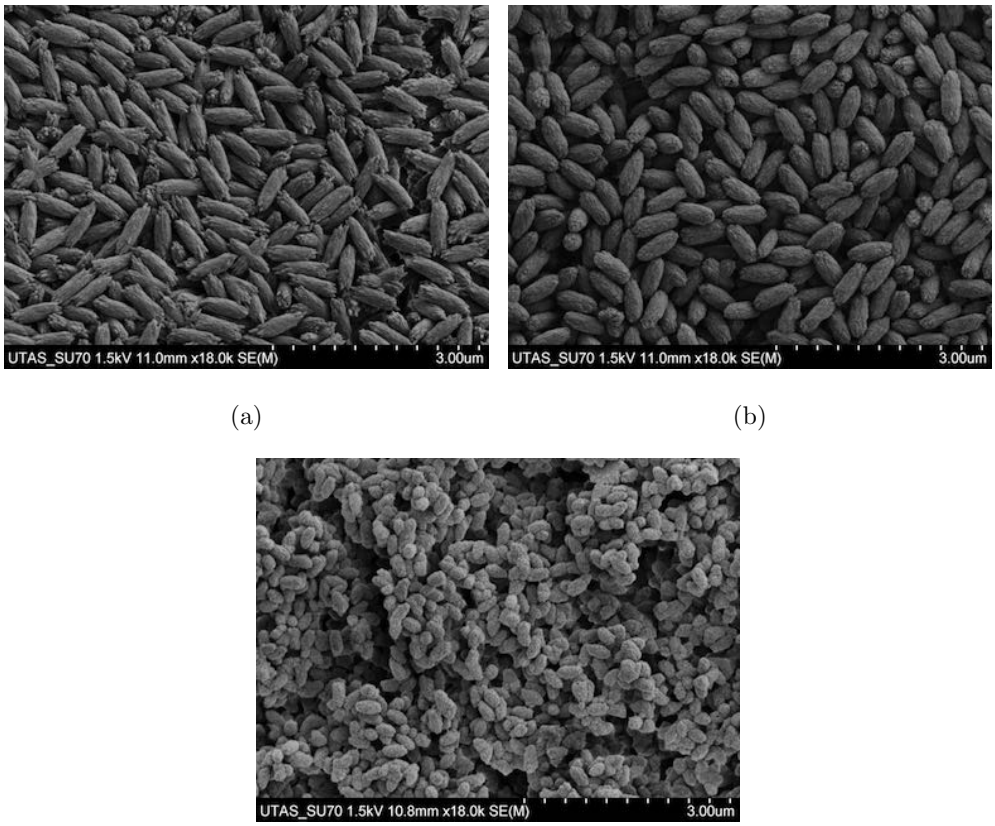
It was found also that the difference in regularity between a buffered gel and unbuffered was slight. If the pH of the gel was set between 5.5 and 6.5, then the addition of a buffer had only a minor effect. These findings were not reported elsewhere.

4.3 The use of carbonate buffers in the gel

As shown in Fig. 4.2, it may be possible to use a buffer system to reduce the volatility of the gel pH as it was heated and as the reaction proceeds and thus increase the regularity of the particles produced. It may also be possible to reduce the overall drop in pH as the temperature increases, which again might improve regularity and provide additional control over the shape of the particles produced.

When added to the gel, ammonium carbonate or ammonia bicarbonate can act as a buffer. As the solution was heated it would be expected that the ammonium carbonate would decompose into the ammonium bicarbonate and then decompose into ammonia and thus the ammonium carbonate could possibly offer greater control than the bicarbonate [12]. To test this, as shown in Fig. 4.3, each carbonate was added to a gel and a precipitate produced.

Instead, it was found that adding a carbonate, shown in Fig. 4.3(a) at pH 5.2, and in Fig. 4.3(b) at pH 6.0 and in Fig. 4.3(c) at pH 6.8, decreased crystallinity and particle uniformity. The carbonate added possibly formed carbonated apatite, which is where the $[PO_4]^{3-}$ anion in the apatite is replaced with $[CO_3]^{2-}$.



(a) (b)

(c)

FIGURE 4.3: The addition of a buffer containing carbonate to the solution gel reduced the regularity and crystallinity of the particles produced. The precipitate produced from a carbonate buffer set to a pH 5.2 (Fig. 4.3(a)), 6.0 (Fig. 4.3(b)) and 6.8 (Fig. 4.3(c)) is shown.

The X-ray pattern supported various levels of crystallinity are listed in the draft paper in the Appendix A. Due to the constraints of the target journal's layout, these images are rather small. The work crystallinity and geometrical shape of particles should not be used interchangeably. They are different physical properties. This point should be clarified in the main text.

It was also found that the bicarbonate was better than the carbonate at producing regular particles, possibly because the particles were less carbonated. No increase in regularity was observed due to the proposed increase in control over solution gel pH. Carbonated apatite has been reported elsewhere [12].

This result suggests that having a carbonate in the gel may provide some buffering,

but this potential advantage was negated by the carbonate incorporating itself into the apatite [12].

4.4 A comparison of preheating the gel vs. ramping its temperature

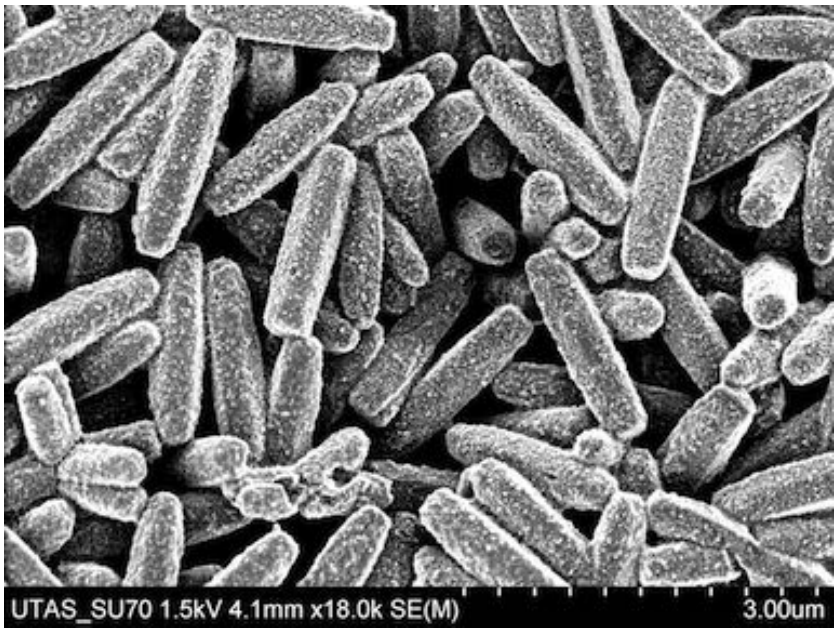
The most common approach to incubating the gel was at a fixed temperature in a preheated oven, on a hot plate or at room temperature on a bench [7]. One report suggested, however that the rate of heating and cooling the gel might be worth considering [21], although in this report it was not used to control the morphology of the precipitate.

The solution gel with an EDTA chelate was slowly heated for 4 hours at 0.6 °C to 160 °C and then rapidly cooled and its precipitate was compared to precipitate formed after incubating in a preheated oven for 4 hours at 160 °C. As can be seen in Fig. 4.4(a), the gel incubated at a fixed temperature produced particles that were regular, but some variance in size can be seen. In Fig. 4.4(b), the particles produced from a gel the was heated slowly appeared more regular in their shape and size. Ramping the incubation temperature of the gel appeared to increase the particle regularity. This finding was not reported elsewhere.

A limitation of these tests was that with a slow or high rate of heating, the incubation time (at a high temperature) was either shorter or longer. It was difficult to untangle which parameter, the rate of heating or the length of the incubation, was having an effect. Previous tests suggest that a four hour incubation at a fixed temperature was sufficient (to achieve a crystal precipitate), and so to match this length of time, a heating rate of 0.6 °C per minute and a final temperature of 160 °C was chosen.

This result was also seen when citric acid was used as the chelate in the gel. Possibly the slight increase in regularity observed after the gel was slowly heated, reflects a more controlled and slower release of the calcium ions from the chelate molecules in the gel [30].

(a)



(b)

FIGURE 4.4: Ramping the temperature of the solution gel slowly and in a controlled manner from room temperature to a final temperature rather than placing it in a preheated oven, increased the regularity of the particles produced. Particles were produced after incubation in a preheated oven (Fig. 4.4(a)) and after incubation in an oven whose temperature was ramped (Fig. 4.4(b)).

4.5 The use of oxalic acid as a chelator in the solution gel

Citric acid was the chelate most commonly reported in hydrothermal reactions that form apatite [17, 30] and when it was used in this work, consistent particles were produced. Oxalic acid is similar to citric acid in structure and, it was thought, might therefore have the same effect and so was added to gels as a replacement for citric and tested.

The hydrothermal reaction was run as before, but with oxalic acid added instead of citric and at the same concentration and, in some tests with a 1:1 ratio of water and organic solvent. With oxalic acid generally the particles produced appeared aggregated, irregular, prismatic and flat. The particles were brick-like and intersecting at pH 3.8 (as can be seen in Fig. 4.5(a)). They were less so at pH 4.9 (Fig. 4.5(b)) or if dioxane was added (Fig. 4.5(f)). They were heart-shaped at pH 5.6 (Fig. 4.5(c)). If dimethyl sulfoxide (DMSO) (Fig. 4.5(d)) or dimethylformamide (DMF) were added (Fig. 4.5(e)), they were diamond-shaped.

This shape was not typical for an apatite and cannot be explained by the crystal lattice formed from ions of apatite. **The addition of oxalic acid to the gel provided a new (but irregular) range of particle shapes not seen elsewhere.** The shape achieved is of great interest, but its use may be limited for the intended aim of this work as the particles that were produced were irregular and conjoined and the method was complicated by the toxicity associated with handling oxalic acid.

4.6 The impact of dioxane as a solvent in the solution gel

Just as citric acid can be replaced with oxalic acid, as demonstrated in this work, it might also be possible to use a solvents other than water in the gel. In this test, a one-to-one ratio of water and dioxane formed the gel and the pH was set to 6.0. The

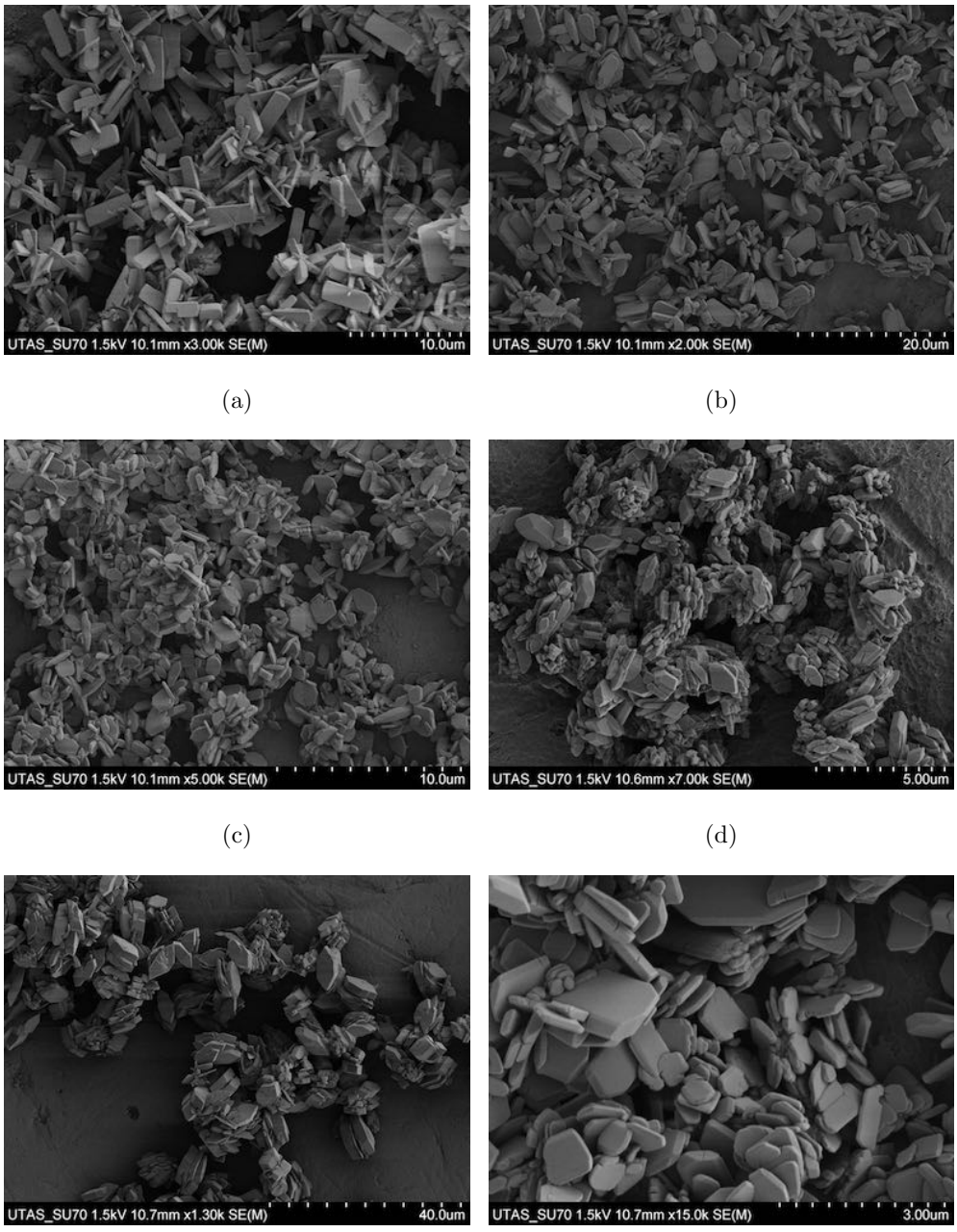


FIGURE 4.5: Oxalic acid in the solution gel produces particles that were bricks-like in shape. The particles shown were formed from a solution gel containing oxalic acid set to a pH of 3.8 (Fig. 4.5(a)), 4.9 (Fig. 4.5(a)), 5.6 (Fig. 4.5(a)) or with gels that also contained DMSO (Fig. 4.5(a)), DMF (Fig. 4.5(a)) or dioxane (Fig. 4.5(a)) solvents at a ratio of 1:1 with water.

precipitate produced is shown in Fig. 4.6 where dioxane was tested with citric acid, citric acid and EDTA or no chelate added to the gel. The particles produced had an unique gull-wing shape not reported elsewhere. The most regular particles were made with dioxane in the gel with citric acid added or when no chelate was added.

In the Fig. 4.6(a) micrograph, the flat sided gull-wing shaped irregular particles shown were produced with citric and EDTA. In the Fig. 4.6(b) micrograph, the same gull-shaped irregular particles shown were produced, but with tapered rather than flat sided with just citric acid. In the Fig. 4.6(c) micrograph, when EDTA was added without citric, the particles shown lost some of their flatness and regularity. In the Fig. 4.6(d) micrograph, with oxalic acid instead of citric, the particles shown become less regular and appeared more aggregated.

As a control, dioxane was also added without a chelate. As shown in Fig. 4.6(e), tapered more regular, but still intersecting wing-shaped particles were produced. The use of dioxane did complicate the synthesis as it is toxic and the particles produced were irregular and conjoined and would be difficult to assemble.

As shown in other tests reported this work, the shape of these particles defies what is thought possible in apatite precipitate. This implies that the underlying dynamics of their growth might still not be fully understood. The results were limited to tests done with gels at pH 6.0. It might be useful to test these additives at other pH settings.

4.7 The use of evaporation to align nanoparticles

It was beyond the scope of this current work [25, 26], but as it was considered important [24], some alignment of the particles was tested. It was observed in the micrograph Fig. 4.7 that when rod-like particles in water were left to dry on a stub for SEM, they became aligned for at least one micron in length, as a result possibly of capillary forces acting between the particles as the solvent was removed.

This observed short-range alignment of particles was reported elsewhere [26, 43]. For this test, the rod-shaped particles aligned were first produced by a conventional hydrothermal method. They were then washed in ethanol and allowed to dry on a SEM

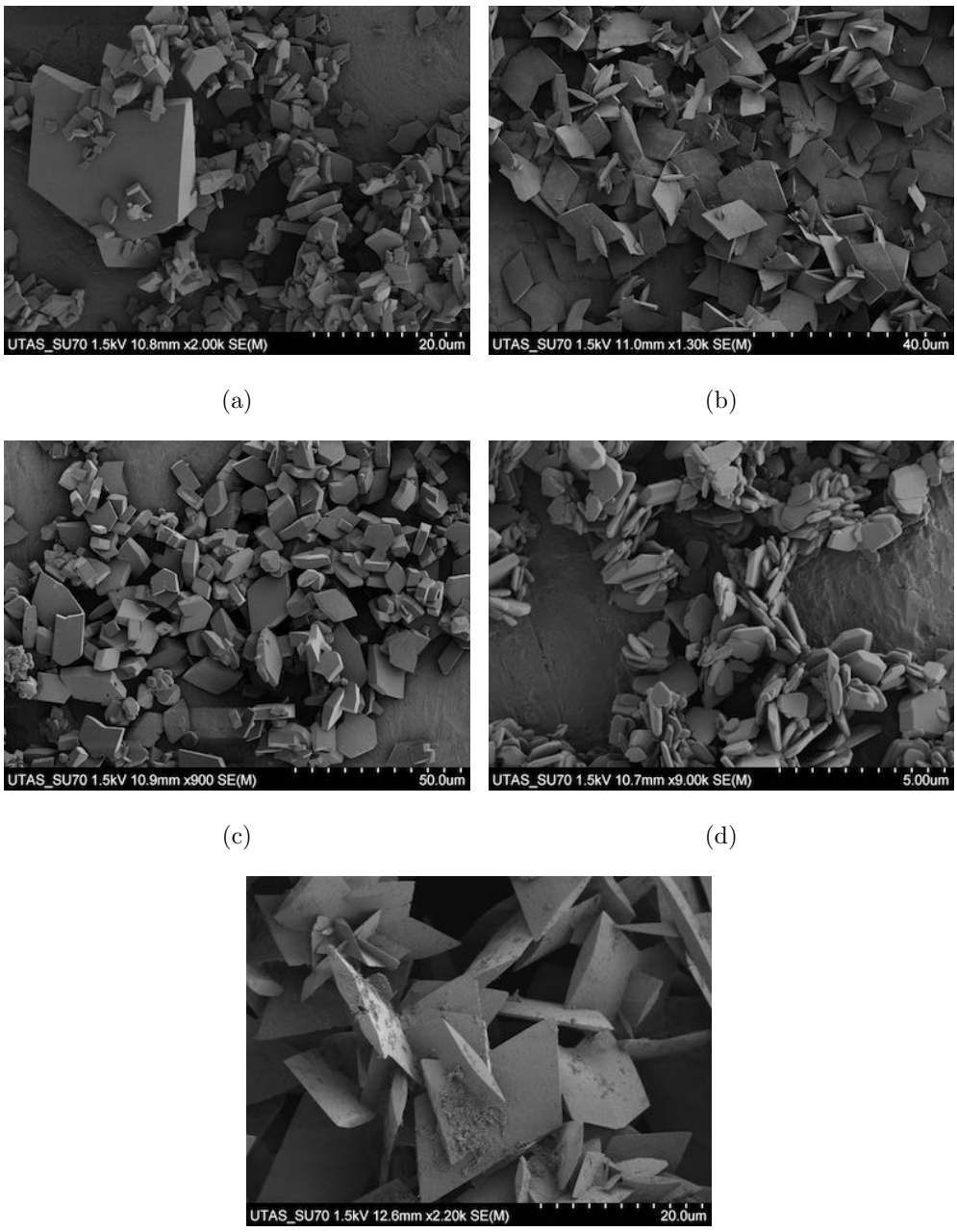


FIGURE 4.6: Dioxane added to the solution gel produced particles that were uniquely gull-wing in shape. Particles were produced from solutions also containing citric acid and EDTA (as can be seen in Fig. 4.6(a)), with citric acid alone (Fig. 4.6(b)), with EDTA (Fig. 4.6(c)), with oxalic acid (Fig. 4.6(d)) or without any additional additives (Fig. 4.6(e)).

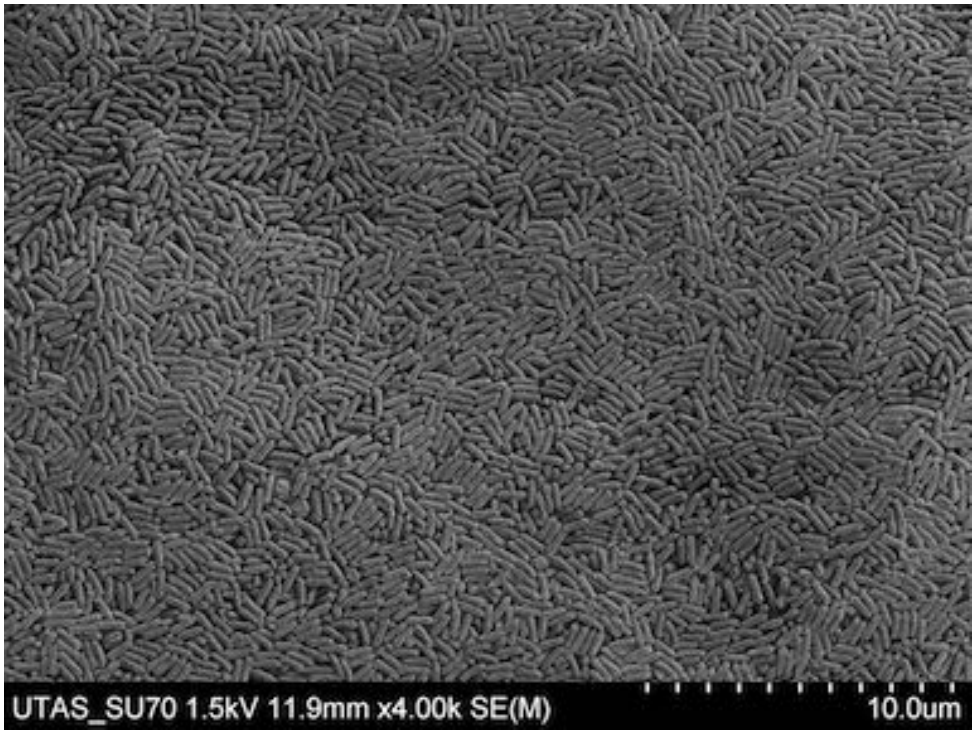


Figure 4.7: When particles in solution were left to dry they can form short-range alignment. No long-range alignment were seen.

aluminium stub substrate. No long-range alignment of the particles was observed. Sets of particles became aggregated together along their long-axes and this occurred for an average of 4 particles. This alignment would then stop next to another group of similarly aligned particles, forming as can be seen a hatch-pattern across the field of view.

4.8 The assembly of rod-shaped particles in the shape of a ball

The alignment of the particles, a crucial step in their effective use in a tough monolith [24, 25], was not tested in this work due to time constraints. But an interesting and unexpected alignment occurred after washing in water, when rod-like particles were diluted in a polar organic solvent such as butanol, and then left to dry on the bench.

A major determinate in aligning the particles was the evaporation of the solvent,

which exerts an attractive force between particles. We tested if alignment of the particles could be produced at a larger scale. As can be seen in Fig. 4.8, the particles were assembled and formed large and irregular clusters or balls. This was also repeated again with flake-like particles, as can be seen in Fig. 4.9. We were not aware of reports elsewhere which demonstrated this superstructure assembly.

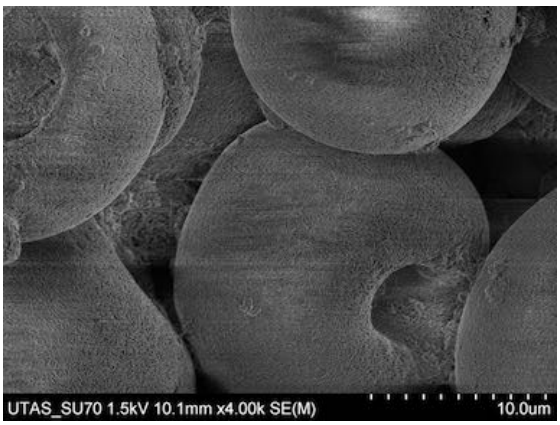
The rod particles were synthesized and then washed in water before suspending in butanol, a non-polar solvent. Possibly the now polar particles, still contained traces of water on their surface and between some particles, aggregated together in the butanol to form regular spheres (as can be seen in Fig. 4.8(a)), which themselves were arranged into the short-range 1 μm wide packing described in this work (see Fig. 4.8(b)) and elsewhere [41]. These long-range 10 μm wide 3D sphere structures contained a plug void at one and were not reported elsewhere. A suggested possible mechanism for this sphere formation is illustrated in Fig. 4.8(c).

We tested if the same approach of first washing with water could also work with irregular plates and with a hydrophilic solvent, ethanol. These plates were first prepared, washed with water and then suspended in ethanol and allowed to dry onto the SEM stub. Irregular spherical particles were again made up of smaller irregular plates (as can be seen in Figs. 4.9(a) and 4.9(b)). These structures were 100 μm wide, or 10 times wider than seen in this work when butanol was used as the solvent. Again this was not reported elsewhere.

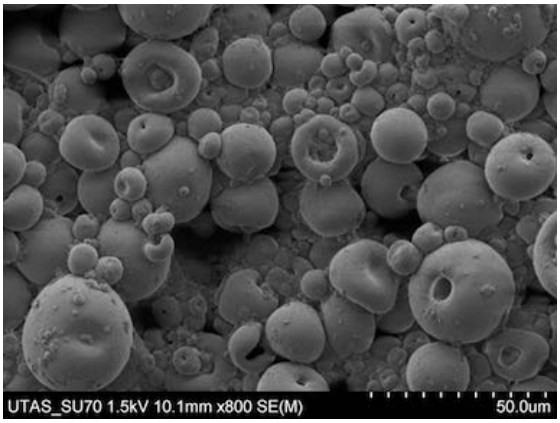
4.9 The difficulty of aligning particles long-range

To summarize this section, when amino acids were added, as described in Section 4.1, the particles formed had a greater regularity and as well the shapes were further modified. While the addition of an amino acid was reported elsewhere [7, 41], the amino acids tested here were different types and here they were tested in the fabrication of fluorapatite.

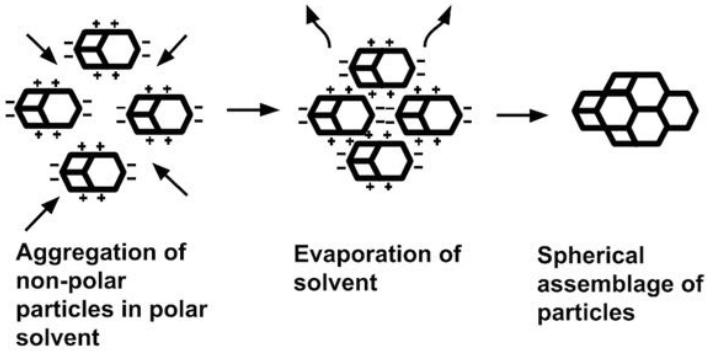
Also in this section, when a buffer was added to the gel, as reported in Section 4.2, the particles became more regular as a result possibly of the pH in the gel became less



(a)

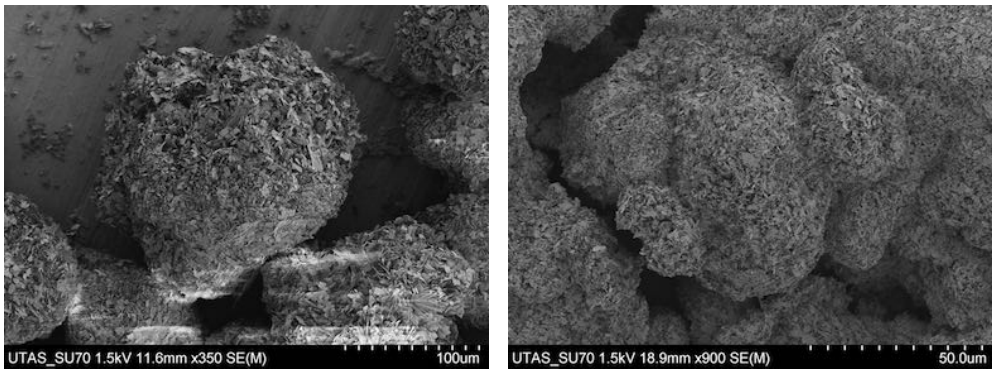


(b)



(c)

Figure 4.8: Particles formed in the aqueous solution gel and then washed with an organic solvent can self assemble into superstructures after this solvent was evaporated. The short-range 1 μm order observed observed in this work is shown (Fig. 4.8(a)). This forms part have a longer range 10 μm diameter 3D packing which contains a plug-like void (Fig. 4.8(b)). The possible mechanism for the assembly of these structures is also shown (Fig. 4.8(c)).



(a) (b)

FIGURE 4.9: Upon the evaporation of an organic solvent, flakes dispersed in this solvent also self assembled into ball-like superstructures. Irregular spheres from irregular plates 100 μm in diameter where observed (Figs. 4.9(a) and 4.9(b)).

varied as the reaction proceeded. Adding a buffer to increase particle regularity was not reported elsewhere. It was also hoped, as observed in Section 4.3, that carbonate added to the gel would further stabilise its pH. Instead particles became carbonated which resulted in less regularity and crystallinity [12].

In contrast to the conventional incubation of the gel in a preheated oven, as shown in Section 4.4, the gel was slowly heated from room temperature to final temperature, before it was rapidly cooled. This was found to increase the regularity of the particles produced and was not reported before to modify shape.

Citric acid was substituted with oxalic acid, as this was described in Section 4.5, and produced crystals that had a brick-like shape, a finding also not reported elsewhere. **Likewise, as observed in Section 4.6, the addition of dioxane with water in the gel produced unique gull-wing shaped particles, a result not seen as well.**

We also reported in Section 4.7 that it was possible to produce some alignment of particles, mostly in the short range. The bigger challenge of arranging particles in the long-scale was also addressed in part by the formation of clusters of these particles into ball and is shown in Section 4.8, but long-range alignment is still a difficult technical challenge to achieve and has yet to be fully resolved in this work and elsewhere [6, 24, 25].

Again, the results in this Chapter suggest that regular particles can be produced by a cut-down optimized version, as seen in Section 3.8, of methods used in the literature. It also demonstrates that commonly added reagents [32] can be replaced with less common ones which were found to still generate particles, but with new and interesting shapes.

An understanding that can be drawn from the results of this Chapter is that a simple gel with a few reagents in it, was more likely than a complex gel with many reagents in it, to produce particles that were regular. The issue of low yield was not addressed in this Chapter. Since it was assumed in this work that it was not enough to produce particles that were the right shape, if large quantities could not also be made, attempts to increase the yield were addressed and are described next in Chapter 5. The yield data is reported in Chapter 6.

5

Optimizing the conventional sol-gel method for making apatite

The focus of this Chapter is to test if it was possible to fabricate regular and complex particles without a complex procedure or a complex gel. This Chapter will describe attempts to simplify the precipitation method, first described in Section 2.4, by using a 40 to 90 °C hot plate and by testing different formulations of the gel. It will take as its starting point, reports in the literature [35, 44] and combine these with the hydrothermal findings, reported in Chapter 3 and in Chapter 4.

5.1 Fabricating regular particles at gel temperatures less than 100 °C

The hydrothermal method developed and described previously in this work required a pressure vessel which had a maximum capacity of 300 mL [33, 34] and used a gas chromatography oven to slowly heat the gel [21]. An aim of this work was to create a useful method that reduced this complexity to a minimum.

A test was run to see if it was possible to produce, under atmospheric conditions, the same particles as those already made hydrothermally. An oven preheated to 80 °C was used to incubate the gel for 4 hours. This was a standard laboratory oven and the temperature of the gel was not ramped.

Surprisingly, the particles made, although less regular, were similar to those made hydrothermally, and are shown in Fig. 5.1. To make these particles, the optimized method for producing rod-shaped crystals was repeated (as was shown in Section 3.10), but at 80 °C instead of at a temperature over 100 °C and without ramping the incubation.

Because of this simplification, the method could be performed in a regular oven or a hot plate without an enclosed high-pressure reaction vessel or with an oven with specialized temperature control. The particles produced were less crystalline or regular than the crystals produced by conventional hydrothermal, but still appeared prismatic. The results of the tests at a pH of 7.0 and 6.0 are shown in Fig. 5.1(a) and Fig. 5.1(b) respectively.

This result contradicts somewhat reports elsewhere [42], which implied that heating above boiling point a gel that was pressured was a necessity. This test and its finding may not have been possible without the work done previously in the work to optimise the hydrothermal method. This result, which repeated the typical hydrothermal method, but without a hydrothermal set-up was not reported elsewhere.

5.2 Seeding the solution gel with pre-made apatite particles

The literature suggested that seeding the gel with small particles of apatite might template larger more regular particles [9]. To test if this was possible, small particles were made using a modified version of the precipitation method listed in Section 2.4.

After preparing and mixing the gel, instead of heating, the gel was washed immediately [10] 50 nm irregular spheres were produced. This precipitate was then added to a new solution gel and the precipitation method repeated, but this time in full so that it included a heating and incubation step.

The particles produced, which are shown in Fig. 5.2, were slightly more irregular than if tested without seeding. This is not in line with the expected result [9]. Possibly because the seeds prepared were themselves irregular, larger particles formed from the same solution were also not regular.

5.3 Mixing the gel at room-temperature before heating

Following reports in the literature [37], in an attempt to improve the regularity of the particles made with the precipitation method, the calcium and phosphate solutions were preheated before mixing to form the gel. In this work, previous tests had suggested that conditions at the time of first mixing and forming the gel might have a disproportionate effect on the precipitate formed. The results where both reagents or either calcium and phosphate reagents were heated before they were mixed together are shown in Fig. 5.3.

In agreement with the literature [7], it was found that not heating the precursor chemicals produced the most regular particles. When the chemicals were mixed at room temperature, and phosphate was added to the calcium, the precipitate was crystalline and the particles were regular (Fig. 5.3(a)). If phosphate and calcium were preheated

to 60 °C, and again phosphate was added to the calcium, the crystals produced were irregular (Fig. 5.3(b)).

If the calcium at room temperature was added to the phosphate at 60 °C the crystals were irregular (Fig. 5.3(c)). If the calcium was added preheated to phosphate also preheated to 60 °C, the crystals were again irregular (Fig. 5.3(d)). Preheating the phosphate 60 °C and then adding it to the calcium solution at room temperature did produce larger crystals, but they were also less regular. These results further support the finding in this work that heating the gel up slowly might assist in the formation of regular particles.

5.4 Controlling the speed of addition of the reagents that form the gel

To shorten the method further, it was thought that adding calcium and phosphate together by pouring, which can be done in less than 10 seconds, rather than by dripping with a burette, which can be run for the length of the incubation, would be faster and may not compromise the morphology of the particles produced [44]. This was tested by pouring or by various slower rates of addition with a burette.

Chemicals were added by pouring (Fig. 5.4(a)), added so that addition was complete within one minute (Fig. 5.4(b)) or added slowly over one hour (Fig. 5.4(c)) before running a precipitate reaction for two hours on a hot plate. Little difference was observed between the different addition speeds, possibly because all treatments were then stirred for 1 hour.

This finding was not in agreement with literature, which suggested that the speed of addition has an effect on particle morphology [16]. This literature used an experimental setup, however that differs from the one used in this work.

5.5 Adjusting the amount of mixing of the gel

To further shorten the precipitation method, the mixing component in the method was tested [30]. The gel was not mixed or mixed by stirring for various amounts of time. It was found, and is shown in Fig. 5.5, that some mixing was needed to homogenize the gel and produce regular particles, but there was little benefit to mixing the gel for longer than 10 seconds.

The solution gel was not mixed (Fig. 5.5(a)), or stirred for 10 (Fig. 5.5(b)), 100 (Fig. 5.5(c)), 1000 Fig. 5.5(d)) or 10000 (Fig. 5.5(e)) seconds. Not stirring produced the largest and slightly irregular crystals. It was observed, however that there was little difference between the crystals that were stirred for various lengths of time.

If some irregularity was acceptable, then the gel did not require any mixing and simply adding the reactants together was sufficient to produce a precipitant. If a level of homogeneity was required, then the gel needs to be stirred for at least 10 seconds and for simplicity, stirring the gel for this length of time was considered optimal.

These findings were not reported elsewhere and was in some sense contrary to what was implied in the literature [45], which described mixing by stirring as a separate step or as part of the heating and incubation steps and so was usually run for several hours. **This result suggests that upon its formation, the initial conditions of the gel and whatever treatment was applied immediately afterwards can have a large effect on the morphology of the particles produced. It also suggests that additional processing after this initial period was not required.**

5.6 A proposed mechanism for the formation of apatite crystals

An understanding of how the crystals are formed can be useful as it can suggest ways to control their growth and shape. Formation of crystals with the precipitation method was thought to occur by single monomer addition to a crystallite until a final particle was formed.

A recent report suggests, however that crystals could be formed through a number of routes each acting at the same time in parallel [45]. This mechanism is shown in Fig. 5.6 and was supported by the observations in this work (as can be seen in Section 6.6) which observed that particles appear to be formed and assembled from smaller particles.

Other findings in this work, such as the lack of the expected results seeding the gel in (see Section 6.6), suggest the results from the seeding tests were confounded by the irregularity of the seeds used ([9]) or that the understanding of how this mechanism occurs in this context may not yet be understood.

5.7 The advantages of using the precipitation method to produce apatite

In this Chapter, the hydrothermal method described in Fig. 2.2 was repeated and the results discussed in Section 5.1, but at a lower temperature so that it could be run in an open rather than a closed pressure vessel. Contrary to reports elsewhere, the particles produced were regular, although less so.

This strategy of simplification was then extended by testing a precipitation method, as shown in Section 2.4, with a gel that did not include fluoride or a chelate, and it was found that this greatly simplified method and increased its yield. In Section 5.3, it was reported that regular particles could be produced in large quantity, although they were less regular and complex than those produced hydrothermally with a chelate. The yield data is reported in Section 6.7.

In disagreement with the literature [9], in Section 5.2, it was shown that seeding the gel with small particles of apatite did not improve the formation of regular larger particles. It was expected that small particles would template larger regular particles, but instead irregular particles were produced.

Again in agreement with the literature [7], preheating the precursor chemicals before they were mixed, as described in Section 5.3, did not improve the regularity of the

particles. This result also confirmed that heating the gel slowly rather than using a preheated oven improved the regularity of the crystals produced [21].

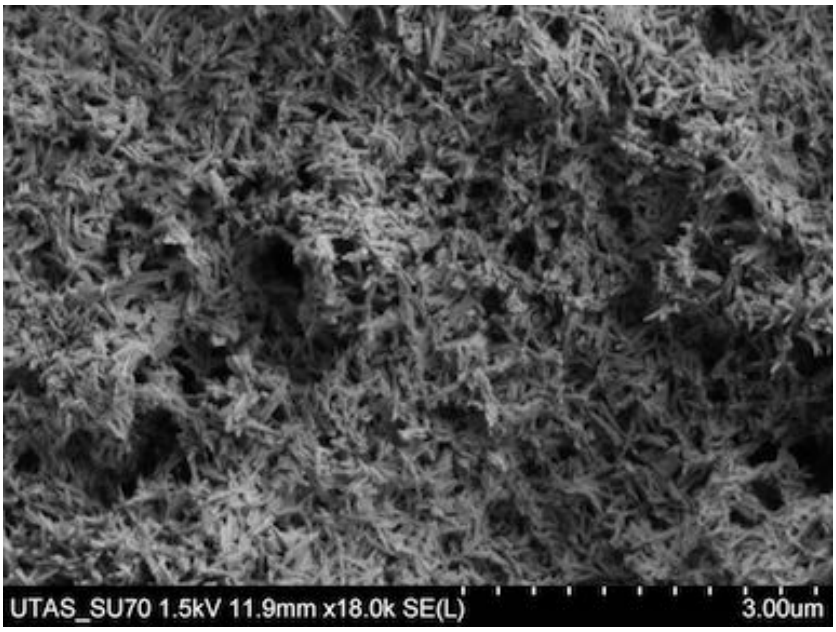
Other findings in this Chapter improved aspects of the precipitation method itself. Pouring the agents together rather than slowly dripping one into the other, was described in Section 5.4 as being simple to perform and did not compromise the product of the reaction [44]. It was also described in Section 5.5 that shaking the gel rather than stirring it simplified that step and made the method slightly shorter [30].

These improvements to this method demonstrated that it could be run simply and produce a high yield of particles with a uniformity (as defined in Chapter 2). What was not the focus of this Chapter was the turnaround time of the method. The precipitation method could be completed in two hours, but the results were optimal if it ran for four. This was short compared to methods reported in the literature [31], but was still much longer than 1 hour, which was one of the aims of this work. Shortening this time is reported next in Chapter 6.

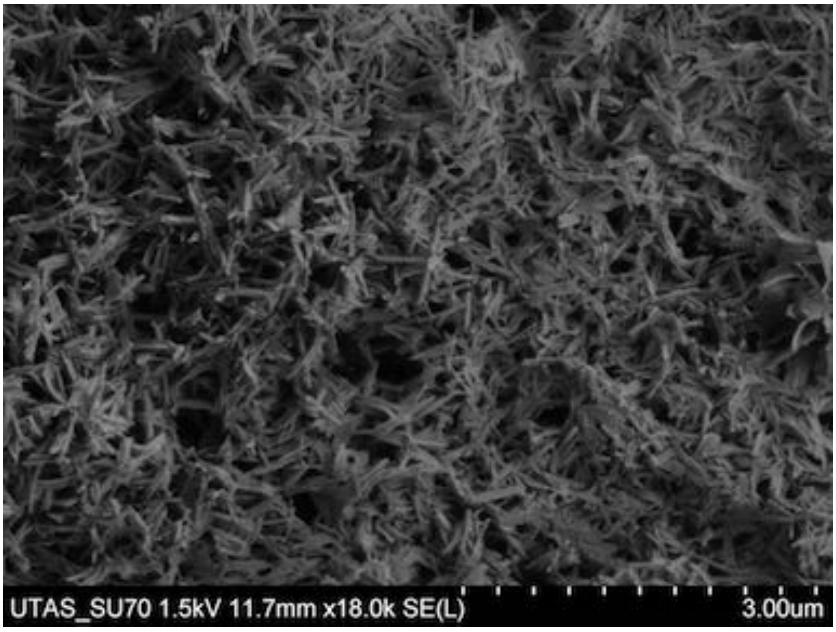
(a)

(b)

Figure 5.1: The hydrothermal method typically run at high temperature, was repeated at a temperature below boiling point. This new method was performed at a pH of 7.0 (Fig. 5.1(a)) and 6.0 (Fig. 5.1(b)).



(a)



(b)

Figure 5.2: The solution gel was seeded with pre-synthesized nanoparticles of hydroxyapatite. After incubating at 60 (Fig. 5.2(a)) or 80 °C (Fig. 5.2(b)), the particles produced were irregular in shape.

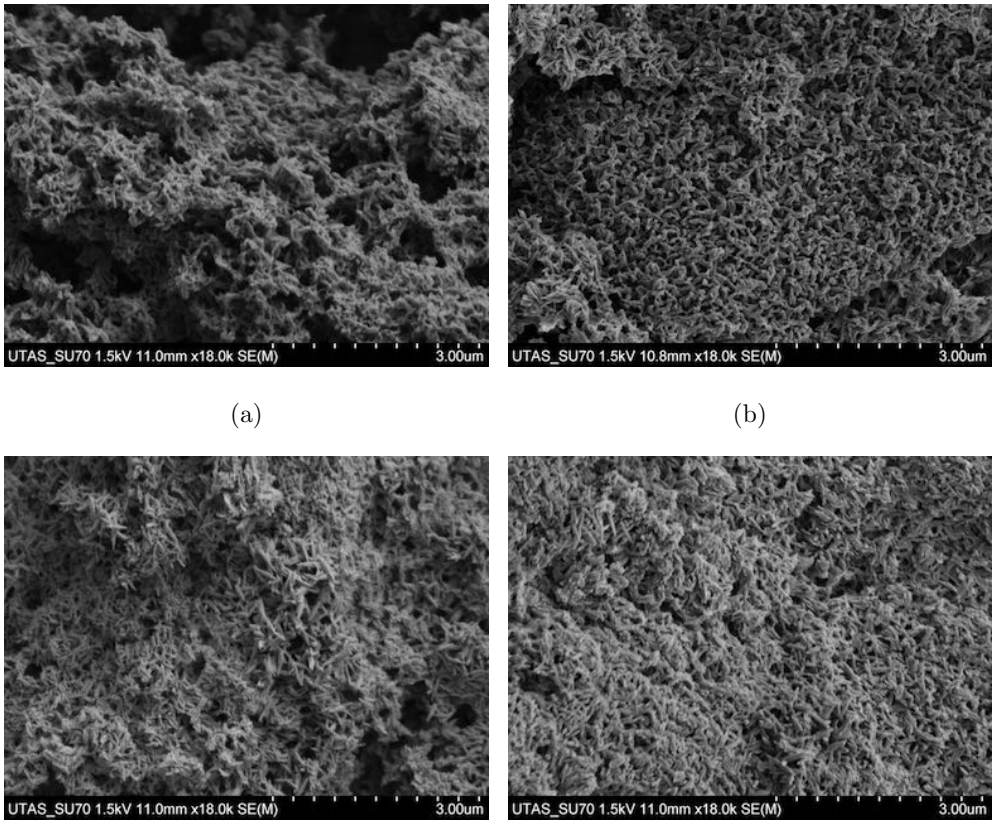
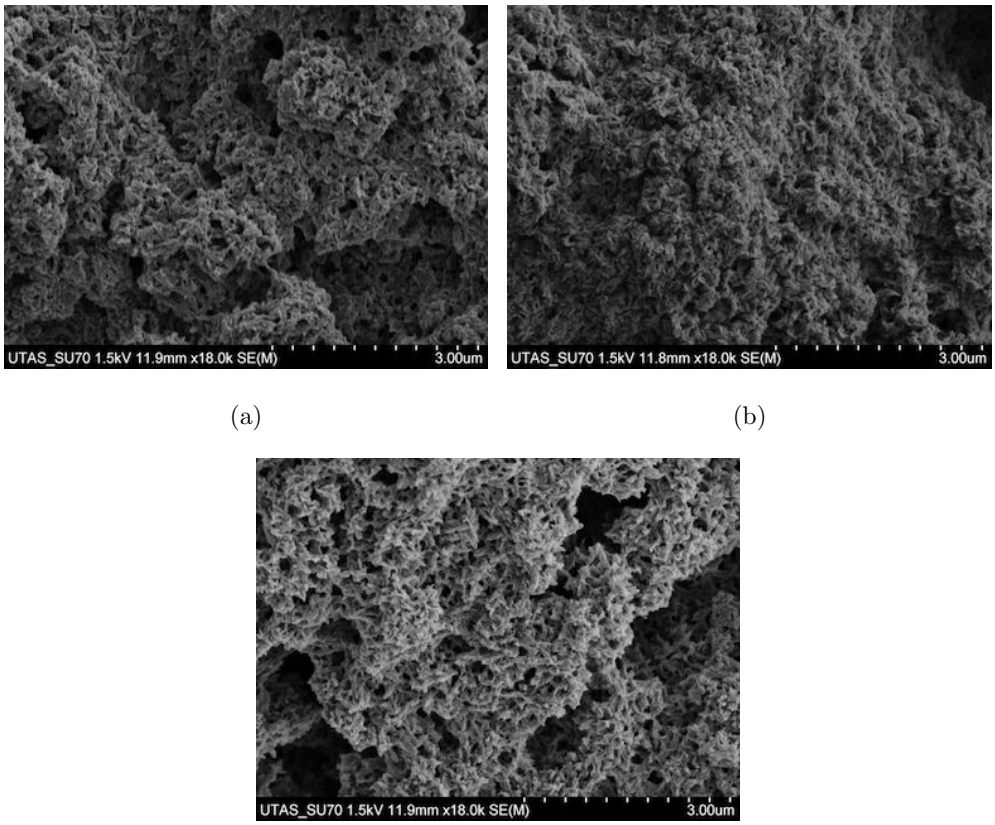


Figure 5.3: Solution gels that were prepared and mixed at room temperature produced the most regular particles. Particles produced from room temperature prepared gels (Fig. 5.3(a)), gels prepared from preheated to 60 °C calcium and phosphate precursors (Fig. 5.3(b)), those where the phosphate precursor was preheated (Fig. 5.3(c)) and when the calcium was preheated (Fig. 5.3(d)) are shown.



(a) (b)

(c)

Figure 5.4: Pouring was simpler and faster and produces the same precipitate as a burette. The solution gel was prepared by pouring a phosphate solution into a calcium solution (Fig. 5.4(a)), doing the same, but over a period of 1 minute (Fig. 5.4(b)) and again, but over 1 hour (Fig. 5.4(c)).

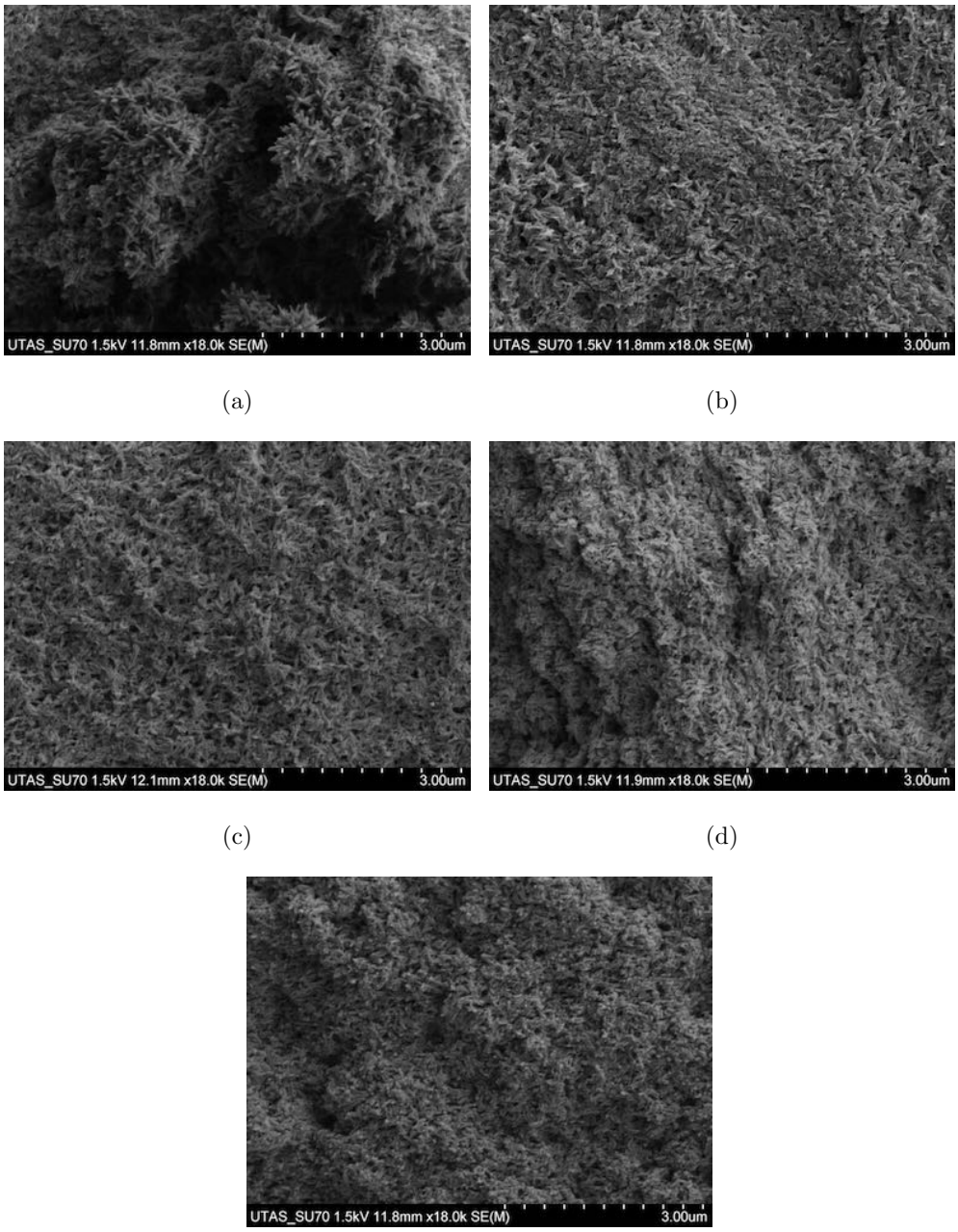


Figure 5.5: Some mixing of the solution gel was necessary to produce a homogeneous precipitate. The gel was either not mixed (Fig. 5.5(a)), or stirred for 10 (Fig. 5.5(b)), 100 (Fig. 5.5(c)), 1000 (Fig. 5.5(d)) or 10,000 seconds (Fig. 5.5(e)).

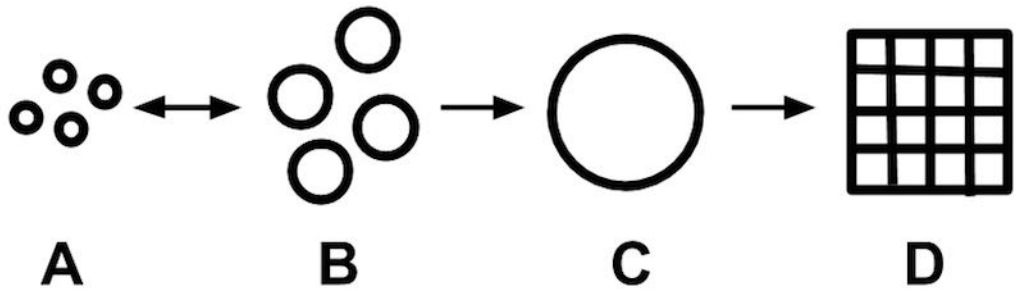


Figure 5.6: A proposed mechanism for the formation of crystals of apatite is shown. The ions in solution can aggregate (A) and then further aggregate into clusters (B). These clusters can then densify and nucleate (C) to form crystals (D).

6

The development of a novel microwave method for making hydroxyapatite

Apart from running many reactions in parallel, the amount of precipitate can be increased indirectly by shortening the method. It can then be repeated many times. Reports elsewhere suggest that using a microwave to heat the solution gel instead of using a hot plate or an oven might be much quicker [19].

For many laboratories, a microwave is still not standard equipment. A commercial system can be expensive to acquire and might only be able to process a small volume [18]. This is not true for the common domestic microwave, which can be cheap to acquire, easy to run and can process up to 400 mL of solution gel per batch.

There may, however be some downsides to using a household microwave. For instance, compared to a hot plate or a commercial microwave, there is no fixed temperature control [18]. Most microwave ovens available today can be set to run at a certain power level for a set time rather than to a temperature.

But this limitation over the control of the temperature in the gel may provide an opportunity. Rather than incubating at a fixed temperature for a period of time, a better approach might be to heat for a period of time to a temperature maximum and them cool. This approach was much like the temperature ramping tests reported earlier in this work that used a hydrothermal approach.

6.1 The use of microwave to rapidly heat the solution gel

In this work it was found that regular particles can be made hydrothermally but the shortest incubation time was 1 hour and the particles produced after this incubation were ill-formed compared to those incubated for at least 4 hours.

Possibly a faster method might be flame synthesis, but this requires specialised equipment and the shapes of the particles produced are limited to spheres [46]. Another approach was to heat by microwaves. Many shapes can be produced quickly using a microwave [7].

To test the shortest time possible to produce regular crystals of apatite, the solution gel was heated for 10 minutes in a microwave and, for a comparison, heated for 10 minutes on a hot plate (and shown in Fig. 6.1). To heat the 8 mL of solution gel over 10 minutes to 80 °C with a microwave, the 8 mL of gel was sealed in a plastic tube and placed in a 100 mL plastic tube filled with water.

Fig. 6.1(a), shows fully reacted and crystalline precipitate after heating with a microwave for 10 minutes. Fig. 6.1(b) shows low crystalline not fully reacted precipitate produced after heating with a hot plate for the same length of time.

The two sets of apatite crystals shown in Fig. 6.1(b) were produced by a hot plate

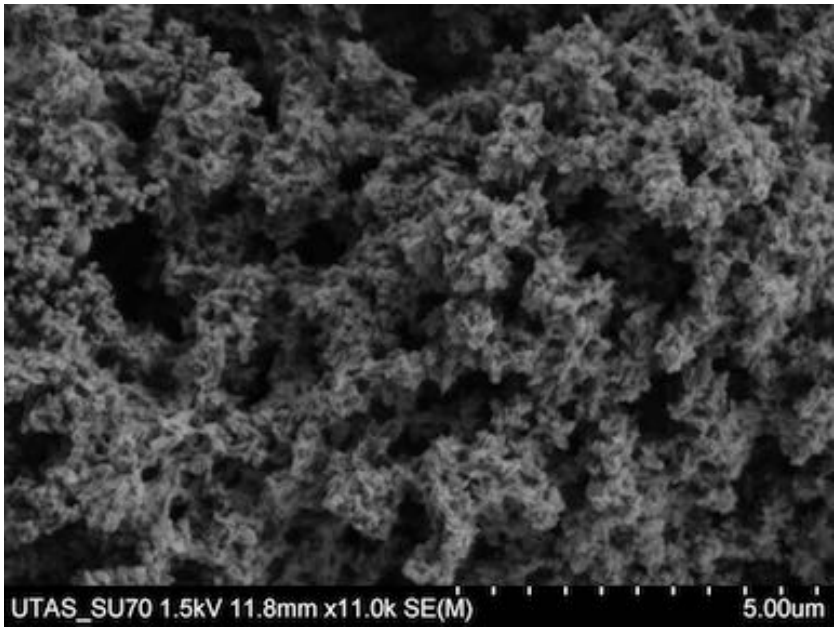
or by a microwave and they illustrate that crystal particles can be produced that are similar in size and shape using either method (see Fig. 6.7(b)), with a small increase in the crystallinity when the microwave was used. However, for the most part, the similarity of their formation underscores the importance of the microwave approach over conventional heating; the product was similar, but it can be completed about 20 times faster in a microwave than when heated on a hot-plate and about 40 times faster if the solution gel was left on the bench to react without heating (see Fig. 6.2).

There were some limitations, however to heating by microwaving. Even if a commercial system was used, the gel temperature that was set was not as stable nor as accurate as the temperature achieved when an oven was used. If an inexpensive domestic microwave was used (as was done in this work) there was further loss of control over the heating rate and final temperature of the gel [13].

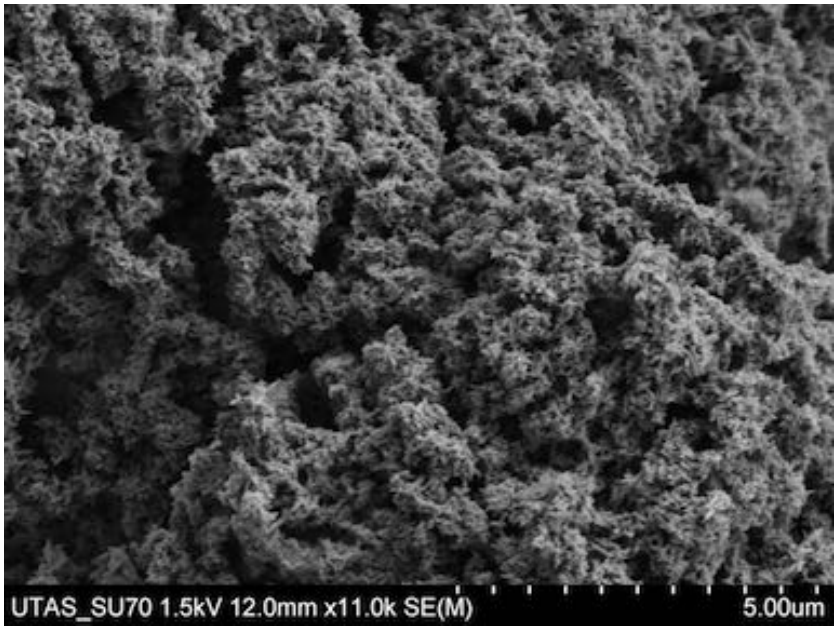
The final temperature to solution gel was heated too was varied. Heating the gel rapidly to 85 °C produced particles that were more crystalline and regular than heating either slowly or rapidly to a temperature above or below that. Temperatures below 65 °C were insufficient for crystal formation, and temperatures above 85 °C were over sufficient; crystal formation was disrupted by bumping.

It was possible to run a domestic microwave within a range of temperatures by simply manually turning on and off over a period of time, but this was too inaccurate. We found instead that it was possible to control the temperature of the gel by heating at a set power and to a set time until a target temperature was reached. The gel can then be removed and left to cool on a bench.

To gauge the temperature the gel was heated to, the time needed to heat a volume of water to a desired temperature at full power was pretested before the gel test was run. In the test reported here, 8 mL of solution gel was microwaved for 10 minutes by enclosing it in a larger 100 mL container full of water. This setup was used so that the gel could be heated for over 10 minutes before its temperature reached 85 °C [4].

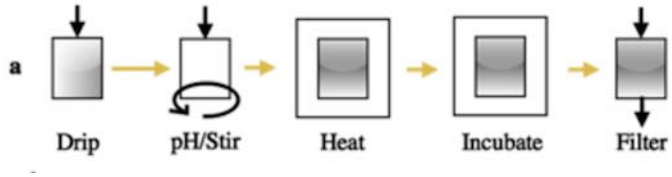


(a)

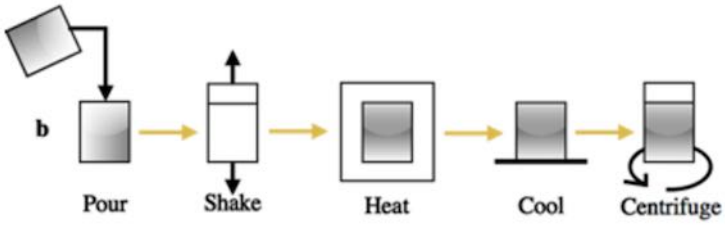


(b)

FIGURE 6.1: The microwave method produced the same crystals as the precipitate method, but took a less time to do so. Crystals were produced after heating the gel with a hot plate (Fig. 6.1(a)) or a microwave oven (Fig. 6.1(b)).



(a)



(b)

Figure 6.2: Fig. 6.2(a) A schema of the procedure for making crystals of hydroxyapatite typically reported elsewhere [19]. Fig. 6.2(b) The 17 minute microwave assay described in this work.

6.2 Managing the regularity of the particles after heating the gel with a microwave

The high regularity of the particles reported in this work made with a hydrothermal, precipitation and a microwave method is illustrated in Fig. 6.3 by showing the precipitate at different magnifications. The synthesis of apatite particles with this degree of regularity was not reported elsewhere.

The highly uniform particles produced indicated that they were monophasic in their nature, and this was confirmed in the analysis and identification shown in Fig. 3.10 for fluorapatite and in Fig. A.1 for hydroxyapatite. In Figs. 6.3(a) and 6.3(b), hydroxyapatite particles produced after running a microwave for 14 seconds at full power (and without fluoride added to the gel) are shown at various magnifications. In Figs. 6.3(c) and 6.3(d), particles are shown at different magnifications and were made with fluoride after a 4-hour hydrothermal method.

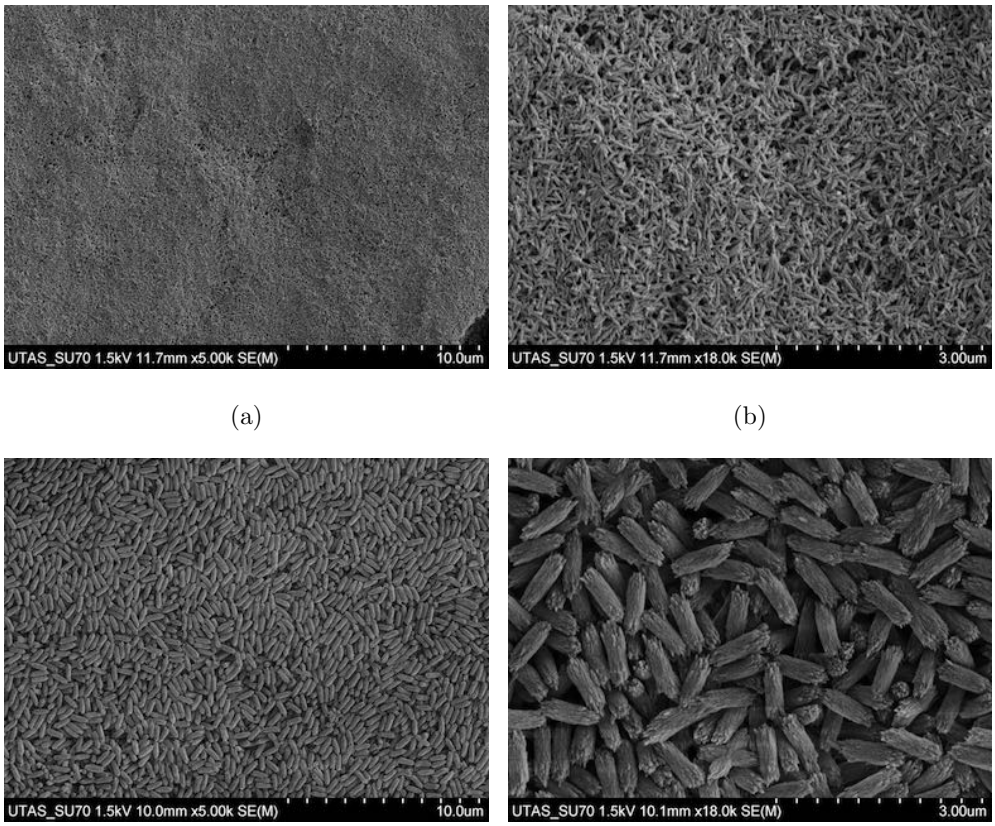


FIGURE 6.3: To demonstrate the high regularity that can be fabricated, particles produced by a microwave (Figs. 6.3(a) and 6.3(b)) and those produced hydrothermally (Figs. 6.3(c) and 6.3(d)) are shown at a low resolution and a high resolution.

6.3 Reordering the pouring, mixing, heating and incubating steps

Tests were run to see if the basic order of the steps common to all the methods reported by the literature and in this work were optimal for making regular particles of apatite [14]. The order listed in the general method - mixing, heating, incubating and washing - and reported in Section 2.1, was tested, and then reordered into as many alternate orders as possible and each time retested.

The common sense steps of adding, mixing, heating, incubation and washing were found to produce the most regular crystals (Fig. 6.4). Yet it

was somewhat of a surprise that some of the variations on this order of steps tested still were able to produce particles with some regularity. However, it would be highly unlikely, if such a fundamental aspect of the method of apatite synthesis was found to not be correct [7].

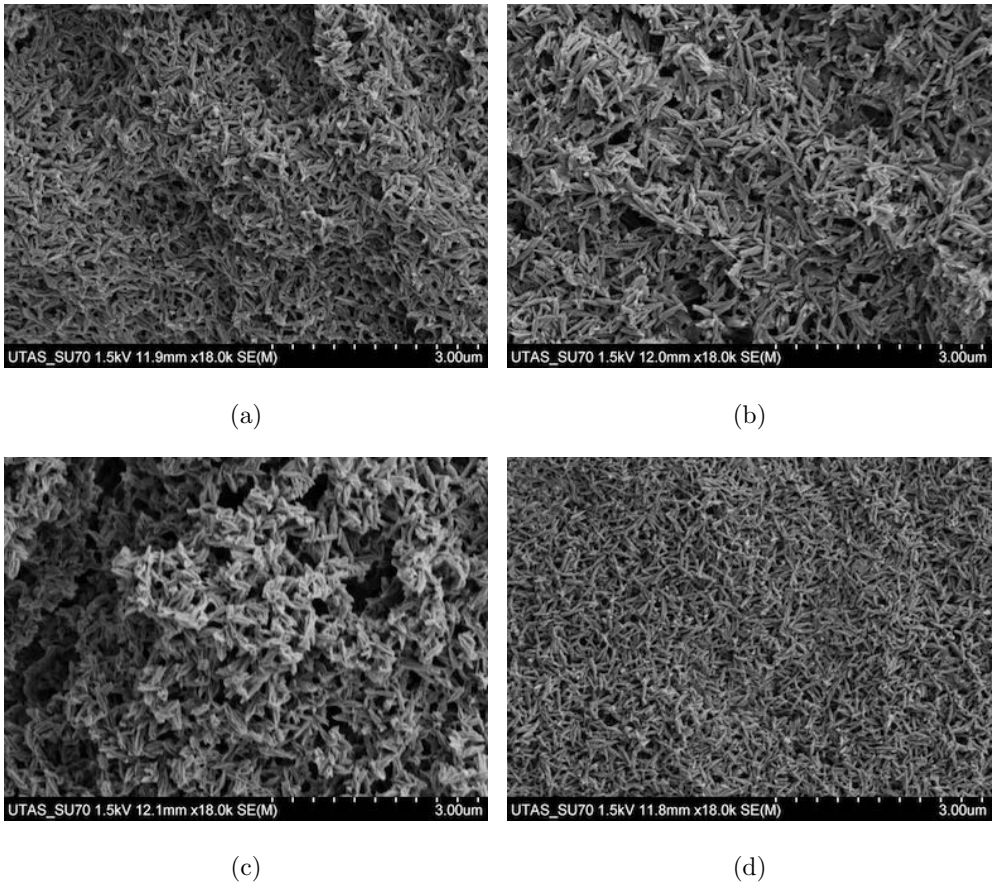


Figure 6.4: The order of the steps in the synthesis methods used - the pouring, mixing, heating and incubating steps - unsurprisingly has an effect on the regularity of the crystals produced. The precursor chemicals were first heated, poured together, and finally the gel was mixed and before incubating (Fig. 6.4(a)). In another test, the chemicals were poured, heated, incubated and then mixed (Fig. 6.4(b)). In yet another test, the chemicals were poured, heated, mixed and then incubated (Fig. 6.4(c)). Finally, the chemicals were poured, mixed, heated and then incubated (Fig. 6.4(d)).

To test the effect the order of these steps can have on the precipitate produced, the typical order (mixing, heating, incubating and washing) was changed. The precursor chemicals were first heated with a microwave for 14 seconds before they were mixed together by pouring and shaking and then held for 10 minutes while the gel cooled

(Fig. 6.4(a)). The particles produced were larger and less regular. The precursor chemicals were poured together, heated with a microwave, held for 10 minutes while it cooled before finally shaking (Fig. 6.4(b)). Again the particles produced were large and irregular.

The chemicals were added, microwaved, shaken and then held for 10 minutes while it cooled. The particles were again irregular (Fig. 6.4(c)). The chemical were poured together, shaken to mix, microwaved for 14 seconds and then held for 10 minutes (Fig. 6.4(d)). The particles were smaller, but regular. This last order of steps was used in this work (Fig. 2.1). This testing outlined here of the order of the synthesis steps was not reported elsewhere.

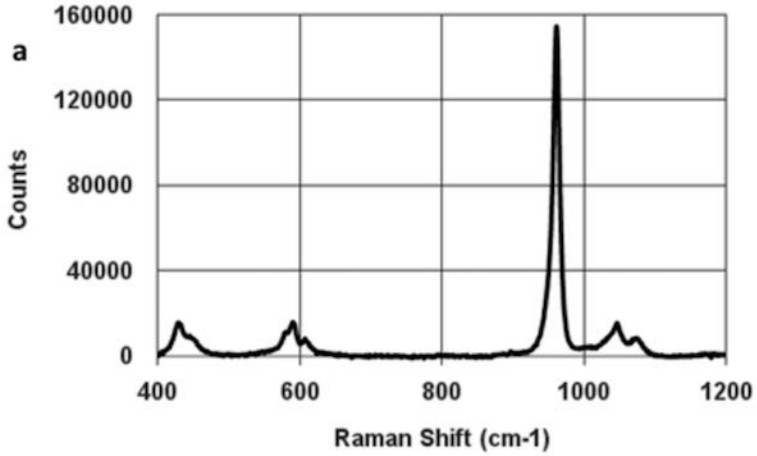
Energy-dispersive X-ray spectroscopy (EDX) was performed with a Hitachi SU-70 field emission scanning electron microscope (SEM) at 7 kV on dried precipitant and the calcium to phosphate weight ratio was measured at 2.1:1, close to the theoretical value of 2.15. For X-ray diffraction (XRD) the dried precipitate was scanned with a D2 PHASER (Bruker), and for Raman, scanned with a Renishaw inVia microscope using a 785 nm laser (see Fig. 6.5). These measurements confirmed that the precipitate was hydroxyapatite.

The precipitate was heated at 10 °C a minute to 1200 °C in nitrogen using a Setaram LABSYS evo system. The precipitate was heated at 10 °C a minute to 1200 °C in nitrogen using a Setaram LABSYS evo system. Thermogravimetric analysis (TGA) showed no loss of weight above 500 °C, which suggests no carbonated hydroxyapatite.

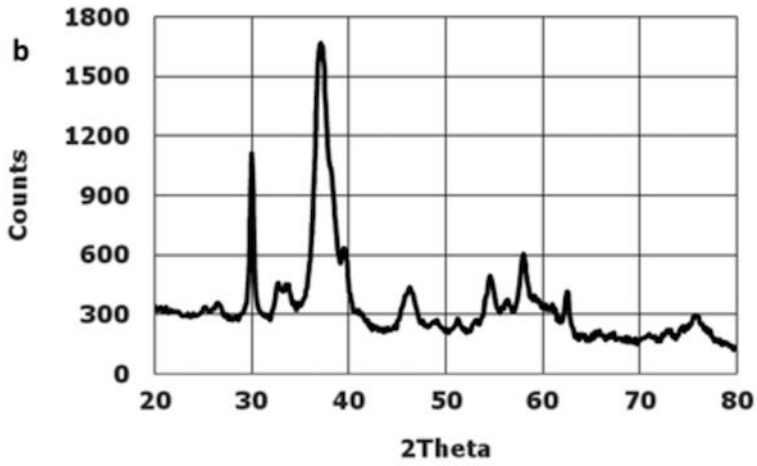
6.4 Testing other ways to mix the gel

To test other possibilities for shortening and simplifying the method, alternatives to mixing the gel were tested [21]. It was thought possible that shaking the reaction vessel would be slightly quicker and simpler if it was found to be as effective at mixing as stirring.

To test this, particles were made after not mixing the gel (Fig. 6.6(a)), or mixing by shaking the gel at 6 (Fig. 6.6(b)), 10 (Fig. 6.6(c)), 20 (Fig. 6.6(d)) and 30 (Fig. 6.6(e))



(a)



(b)

Figure 6.5: Fig. 6.5(a) A Raman spectra and a Fig. 6.5(b) XRD spectra of the precipitate first shaken at 10 times a second for 10 seconds.

times a second for 10 seconds. This interval was observed in the work as slightly longer than the minimum of 6 seconds needed to homogenise 8 mL of gel.

For imaging, the SEM was run at a 2.5 to 40 K magnification and 1.5 kV acceleration. For the zeta potential (calculated at -18.3 mV) and size, a Zetasizer (Malvern Nano ZS Zetasizer) tested precipitate in neutral water. The wet aggregated particle was 1 μm in length, longer than the approx. 0.5 μm observed in the dry precipitate with the SEM (see Fig. 6.6).

It was already determined in this work (Fig. 5.5), that as long as there was at least 10 seconds of mixing, mixing after that had no effect. Similarly, it was found in this test that shaking the gel at any speed made the particles regular. If the gel was shaken faster, the particles produced appeared to be smaller. This test was not performed elsewhere. It was also unexpectedly found that a mechanical shaker was not needed. The gel could be shaken by hand for ten seconds and still produced the same result.

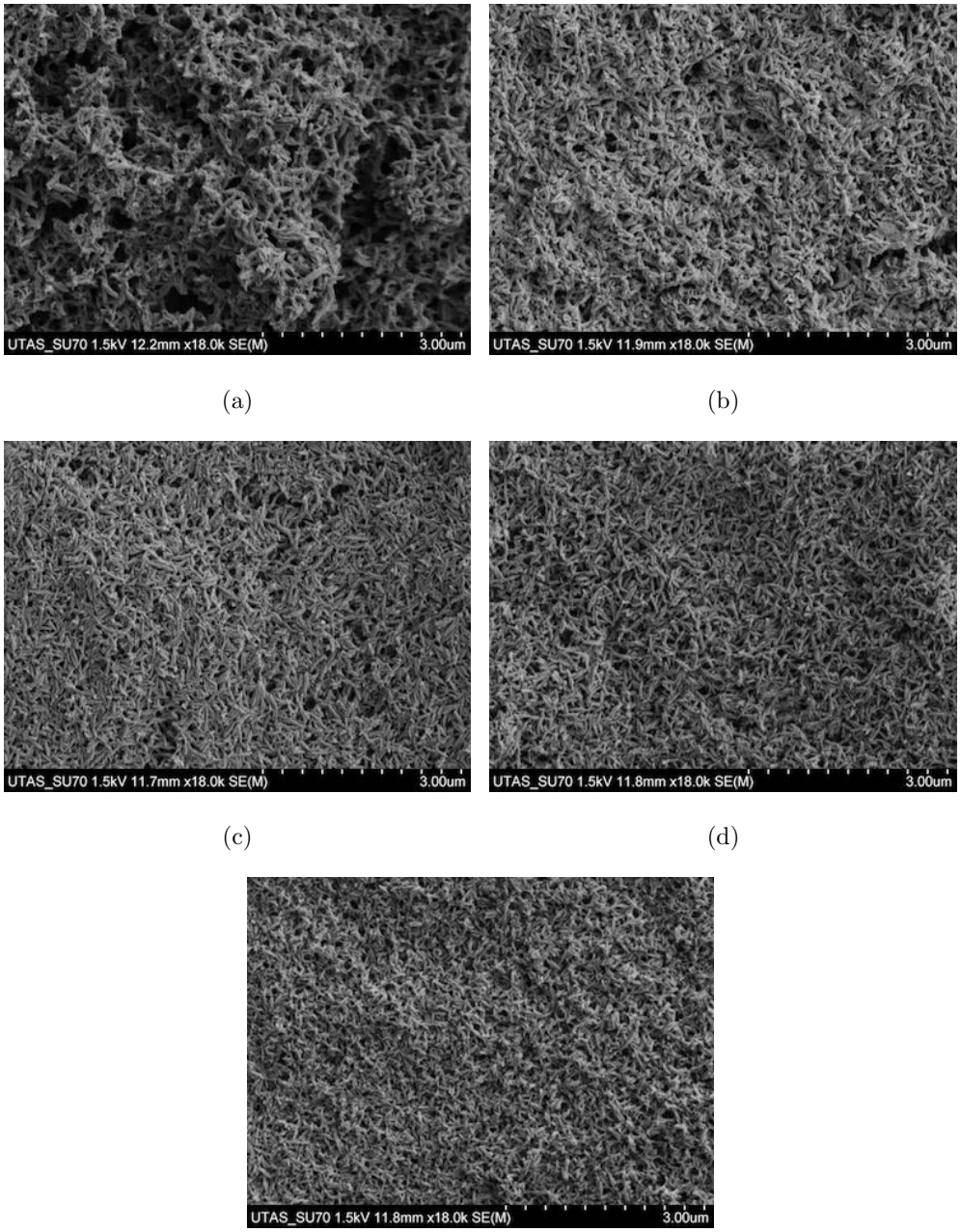
These results were also repeated with a 80 mL container of gel. The reaction was completed in 17 minute with an 8 ml volume and, for the 80 ml volume, in 40 minutes. The same crystals were produce, which suggests the method is scalable. The turnaround time for the 80 ml was shorter reports elsewhere that processed the same volume. In order to do investigate the effect of which reagent to add to which, the order of adding reagents was varied as well as the speed of their addition. Pouring the phosphate into the calcium produced the most regular and crystalline particles in the shortest possible time. It was also found that it was not necessary to preheat the reagents.

6.5 Statistical analysis of particle size

In order to investigate the effect of how the gel was mixed, the solution was either shaken or stirred. Without at least some mixing, the crystals produced cannot be regular, but we found that the gel could be sufficiently, homogenised by shaking and this was much faster. If the gel is shaken instead, mixing can be kept to a minimum and the size of crystals can be kept to a maximum. This finding has not been reported elsewhere.

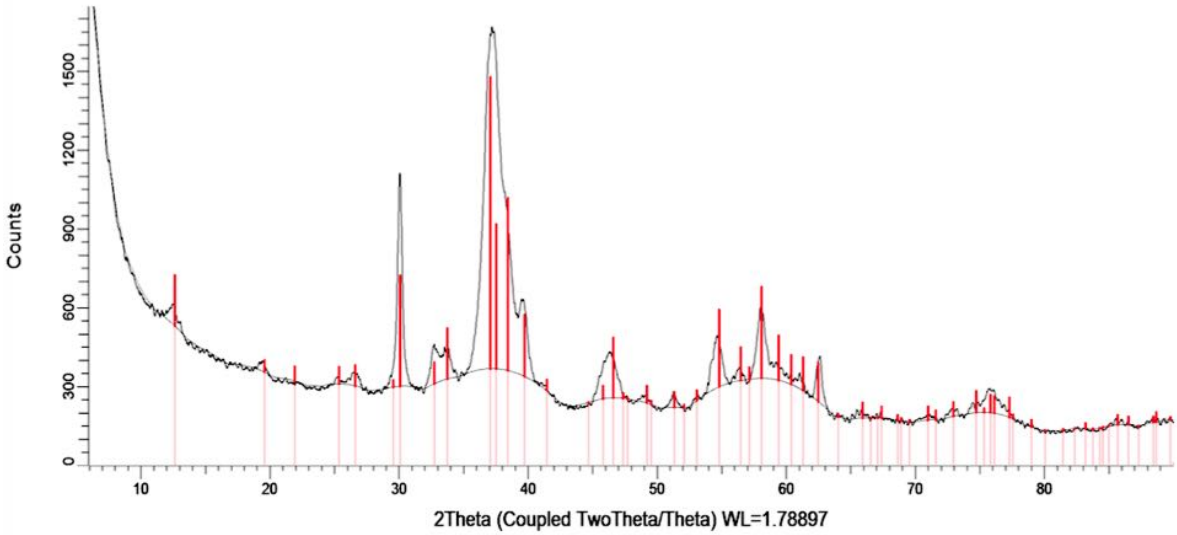
In this work crystals were produced that were as large as 0.5 μm in size (see Figs. 6.6 and 6.7(b) and Table 6.1), which is about 10 times larger than what was reported elsewhere [19]. Shaking can be short, about 10 seconds at 10 times a second to homogenize the solution. At higher speeds of shaking (20 to 30 times a second), although not statistically different, the particles appeared to become smaller.

SEM images were analysed with ImageJ 1.48V (NIH) and SPSS 22V (IBM). Images

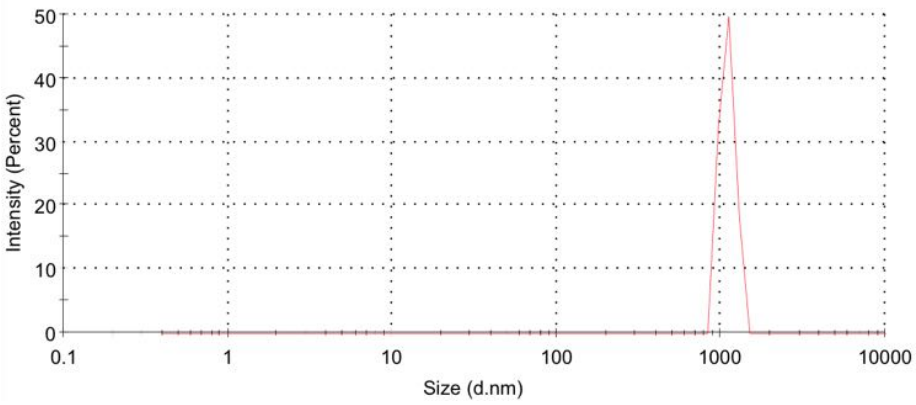


(a) (b) (c) (d) (e)

Figure 6.6: Mixing the solution gel by shaking it rather than by stirring still produced regular particles. The gel was either not mixed (Fig. 6.6(a)), shaken for 6 (Fig. 6.6(b)), 10 (Fig. 6.6(c)), 20 (Fig. 6.6(d)) or 30 times (Fig. 6.6(e)) a second for 10 seconds using a mechanical shaker.



(a)



(b)

FIGURE 6.7: XDR and the particle size distribution analysis of precipitate indicated hydroxyapatite. The results from the XRD indicated hydroxyapatite (Fig. 6.7(a)). The particle size distribution results indicated that the particles in solution were about 1 micron in size (Fig. 6.7(b)).

were converted to 8-bit binary processed with Cell Magic Wand (by Theo Walker). A t-test was run and the mean and 95% confidence interval calculated (n = 30) (see Table). To compare the effect of shaking, a one-way ANOVA (for length, $F(3, 116) = 39.7$, $p = .000$, for width $F(3, 116) = 12.6$, $p = .000$, and for the aspect ratio, $F(3, 116) = 2.3$, $p = .077$) and a Tukey post-hoc test (for not shaking vs. shaking 445 ± 34 nm, $p = .000$) were performed. Normality was tested with Kolmogorov-Smirnov and

Shapiro-Wilk.

Table 6.1: The mean and 95% confidence interval of the length, width and aspect ratio (nm) of the crystals after shaking at different speeds for 10 seconds.

Shaking frequency per second	Mean length of crystal	Mean width of Crystal	Mean length/width ratio
No Shaking	445 +/- 34	165 +/- 22	3 ± 0.4
10	296 ± 28	132 ± 15	2.5 ± 0.4
20	277 ± 24	104 ± 14	3 ± 0.4
30	252 ± 26	106 ± 12	2.5 ± 0.3

6.6 Observations on the microstructure of the particles made with a microwave

A greater understanding of how apatite particles are formed might inform how they can be better made. **An unexpected observation in this work shown in Fig. 6.8 was that the synthesized particles appeared to be assembled from smaller ≈ 50 nm long particles aligned and assembled along the same axis as its parent.** This finding was shown before in the literature [35], but interestingly did not become a focus of investigation.

It is of some interest that most of the micron-sized particles (see Fig. 6.7(b)) shown in this Chapter were formed from smaller particles aligned and assembled together. The Author found throughout this work that most particles were indeed made from smaller particles. In reports elsewhere [35], crystals also appeared to be formed from smaller particles assembled together. It seems in most cases that it is not possible to produce micron-sized particles without also forming these smaller particles except when a precipitate is first formed, not processed further, but instead washed.

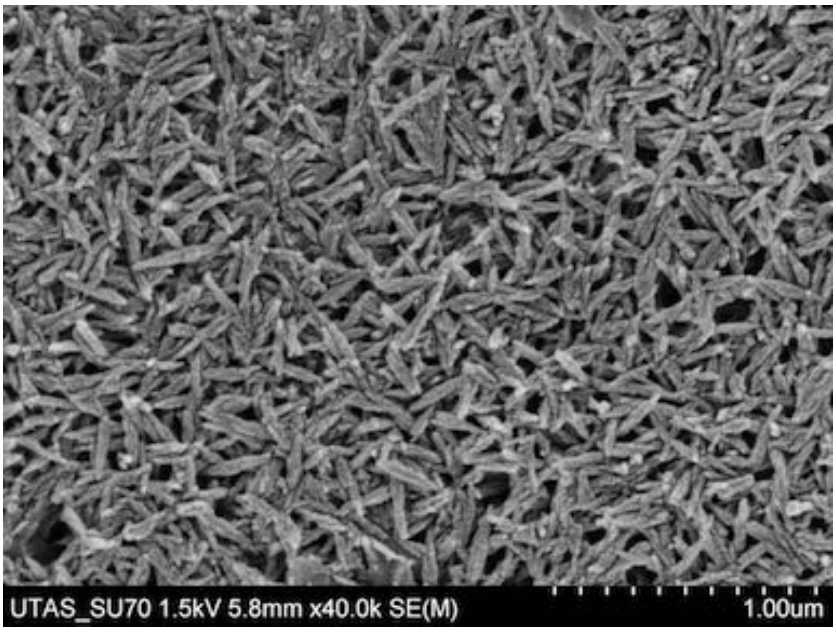
Reports elsewhere describe these smaller 50 nm long particles as amorphous calcium phosphate and not apatite [35], which as mentioned in the introduction, due to structural concerns was not the focus of this work. It is also of note that rarely do these other reports refer to this smaller particle phenomena, even when they were clearly visible in published SEM micrographs. The Author considers the formation of these smaller particles as having some importance. Firstly, their existence may indicate a mechanism for the formation of the larger micron-sized particles into which they assemble. This mechanism has been discussed in this work and elsewhere. Secondly, the formation and assembly into the larger micron-sized particle would have a possible impact on the mechanical properties of these particles.

Although this possibility was not tested in this work due to time constraints, it would be reasonable to assume that structural fracture under load would occur firstly at the interstices of these smaller particles. No data was gathered that suggested that the smaller unit were crystalline. For descriptive clarity in the text here and to be consistent with how it is reported elsewhere [35], the larger particles were referred to in the text as "crystals" and the smaller particles, from which they are made, as "particles". Occasionally the micron-sized crystals are also referred to as crystal assemblies of smaller particles.

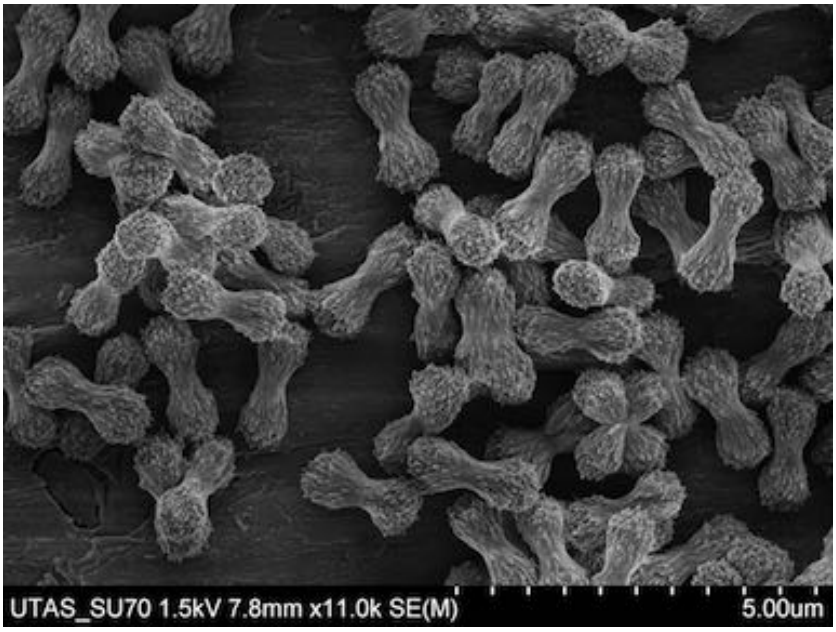
A possible mechanism that might explain this observation is given in Fig. 6.9. It suggests that particles were formed along the same lines as illustrated before in this work (Fig. 5.6) [45].

6.7 Slowly cooling the solution gel after first heating with a microwave

To investigate the effect of a fixed vs. ramped heating and cooling, the solution gel was either heated rapidly and cooled slowly or placed in an oven at a fixed temperature, incubated for 17 minutes and then cooled rapidly in a bath of water. Contrary to results elsewhere, ramping the temperature up and down was as effective as incubating



(a)



(b)

Figure 6.8: The particles produced appear to have been formed and assembled from smaller particles. Both the microwave (Fig. 6.8(a)) and hydrothermal methods (Fig. 6.8(b)) produced particles that appear to be assembled from small particles.

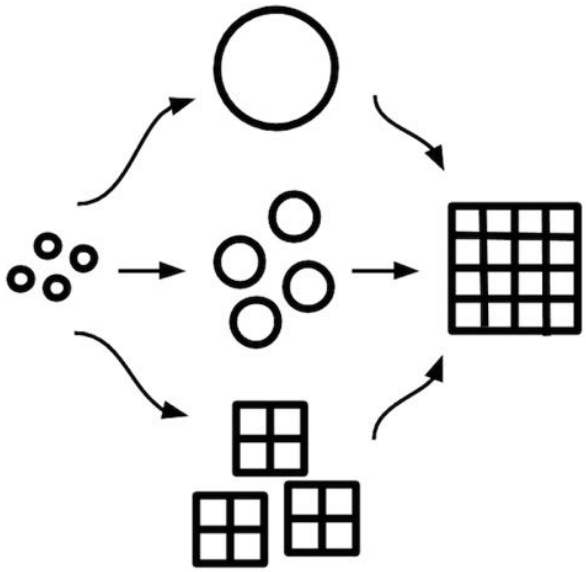


FIGURE 6.9: The results of this work suggest that the particles produced were formed from the self-assembly of smaller particles. (Fig. 6.8).

at a fixed temperature. It was simpler to implement ramping rather then a fixed temperature incubation with a domestic microwave and the crystals were more regular.

To prepare for the method, diammonium hydrogen phosphate ((NH4)2HPO) at 99% purity and calcium nitrate (Ca(NO3)2.4H2O) at 98% purity (Ajax Chemicals) were as received prepared as 1 M stock solutions and then diluted to 0.5 M and their pH adjusted to 10.4 using 16 M of hydroxide ammonia and 2 M of nitric acid solutions.

3 ml of the diammonium hydrogen phosphate solution diluted with Milli-Q (Millipore) water was poured into a 25 ml glass bottle containing 5 ml of the calcium nitrate solution so at a the 1:1.67 stoichiometric ratio for hydroxyapatite. The bottle was sealed and shaken mechanically (Retsch, MM200), typically for 10 seconds at 10 times a second, and then placed at the centre of a turntable (5 rpm) in a microwave oven with the bottle open. This step was also done by hand and produced the same precipitate. The household microwave (Homemaker, EM820CTM) was run at full power until the gel reached 85° after 14 seconds.

Two experiments were then run; in the first, incubation durations of different

lengths were tested. Most methods for the synthesis of apatite incubate the solution gel to a temperature for a time period. **It was found (Fig. 6.10) that if the heated gel was incubated by slowly cooling for at least 10 minutes the particles became crystallised.** An incubation shorter than this, produced particles that were less crystalline and if longer than this provided no further improvement.

To test if the time spent heating and then cooling the gel with a microwave was important, the gels were heated with a microwave for (Fig. 6.10(a)) 6 (or 65°C), (Fig. 6.10(b)) 8 (or 75°C) or (Fig. 6.10(c)) 10 minutes (or 85°C) before cooling for (Fig. 6.10(a)) 10, (Fig. 6.10(b)) 8 or (Fig. 6.10(c)) 6 minutes.

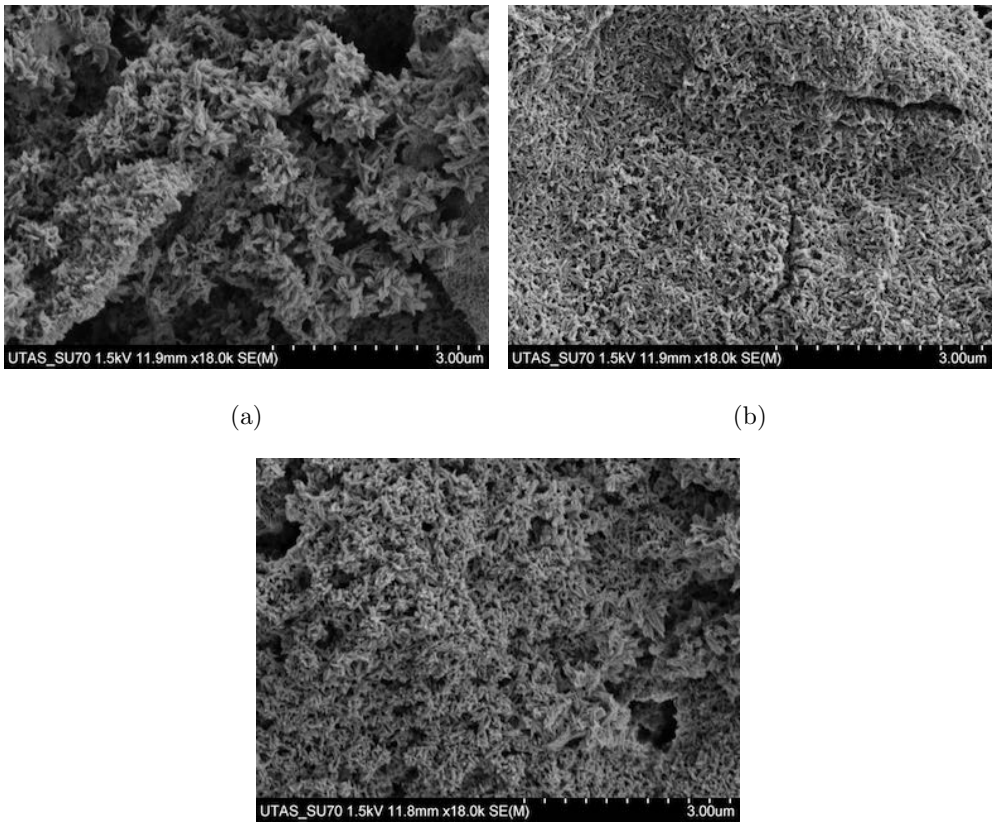
Heating the gel for 6 min. and then cooling for 10 min. produced particles with the most crystalization [40]. It was found, however that crystallinity was dependent on the final temperature the gel was heated to rather than for how long it was heated. It was also found that it was necessary after heating to 85 °C, to cool the gel for at least 10 minutes for crystallinity.

In the second experiment, the gel was heated at full power for different lengths of time, which resulted in different final temperatures in the gel. Heating the gel for 14 seconds, as shown in Fig. 6.11(d), to 85 °C before immediately cooling, by placing the gel on a bench, produced the most uniform crystalline particles [3].

Heating the gel for different lengths of time between 6 to 12 seconds, as shown in Fig. 6.11(a), Fig. 6.11(b) and Fig. 6.11(c), resulted in a lower maximum temperature and produced less crystalline irregular particles.

Heating for longer than this, as shown in Fig. 6.11(e), Fig. 6.11(f) and Fig. 6.11(g) between 16 and 20 seconds to a higher temperature produced irregular particles as the gel began to bump or boil. The crystals are smaller and less prismatic if the solution gel was moved while it is heated. This finding was not reported elsewhere. A full description of the method tested here is also given in Section 6.7.

The results of these two experiments indicate that heating and incubating at a fixed temperature was not needed; rather the gel can be heated quickly with a microwave to a target temperature and then left to cool on the bench, a kinetic treatment that further simplifies the method. It was found that incubating by cooling the gel after



(a) (b)

(c)

FIGURE 6.10: Incubation of the solution gel appears to be necessary for crystal formation at a temperature above 65°C. The gel was heated with a microwave for 6 (Fig. 6.10(a)), 8 (Fig. 6.10(b)) or 10 (Fig. 6.10(c)) minutes. It was then cooled for 10 (Fig. 6.10(a)), 8 (Fig. 6.10(b)) or 6 (Fig. 6.10(c)) minutes on the bench at room temperature.

heating was enough to produce a crystal precipitate and the time taken was much shorter than reported elsewhere [10].

Each experiment was repeated three times and produced a yield of 0.21 g or 84%. The same synthesis was repeated with 30 ml of diammonium hydrogen phosphate and 50 ml of the calcium nitrate reacted in a 100 ml plastic container and produced a yield of 86%. The gel reached 85 °C after 70 seconds of microwaving, cooled to 45 °C after 30 minutes on the bench and was washed at 2000 RPM for two minutes each three times. To calculate the yield, the precipitate was dried in a vacuum (50 mm Hg), washed with water and then 95% ethanol, dried again by suction and then dried at 60 °C to 2 hours under the same vacuum.

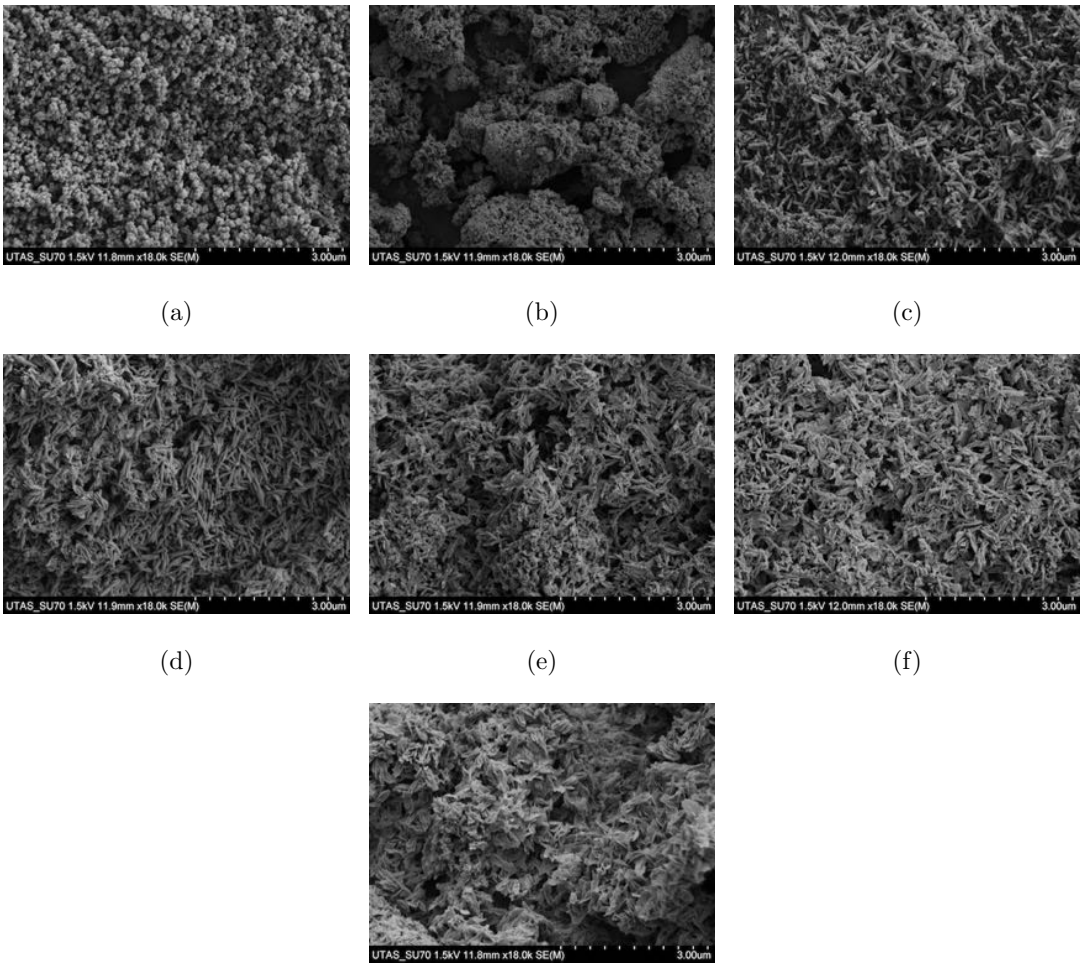
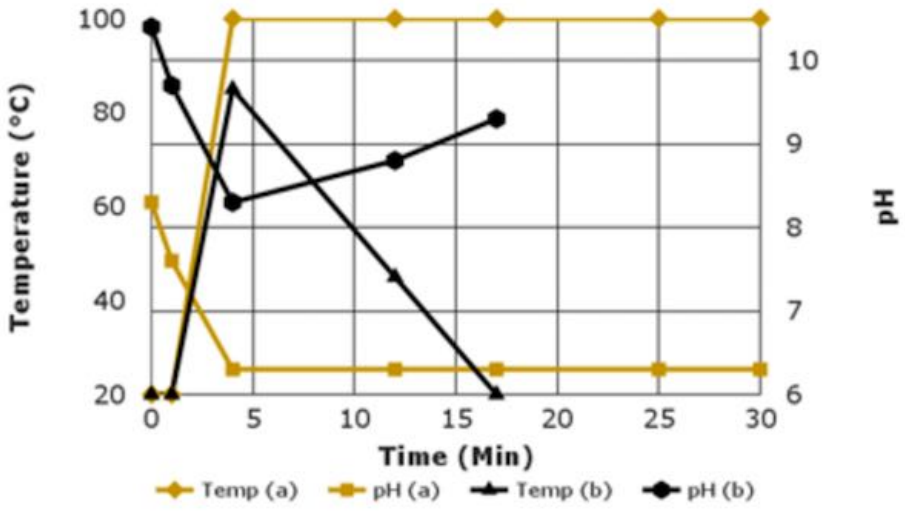


Figure 6.11: The optimal maximum temperature of the gel with tested by heating it with a microwave. The gel was heated at maximum power for 6 (Fig. 6.11(a)), 10 (Fig. 6.11(b)), 12 (Fig. 6.11(c)), 14 (Fig. 6.11(d)), 16 (Fig. 6.11(e)), 18 (Fig. 6.11(f)) and 20 (Fig. 6.11(g)) seconds in the microwave.

These findings suggest that much of what was done elsewhere may not be necessary to make regular particles; incubating at a fixed temperature was not needed; long incubation times was also not needed; and mixing while incubating was not needed. These findings further suggest that conditions at the time of first mixing and gel formation was crucial. If the gel was heated too high or for to short a time, the precipitate was not crystallized.

6.8 Changes to pH of the solution as it is heated and as it cools

In order to investigate the effects of mixing and heating and cooling on the pH, the pH of a gel was measured. The pH dropped from 10.4 to 9.7 after first mixing and then to 8.8 after heating to 85C and finally to a low of 8.3 two minutes after heating before rising to a 8.8 after cooling to 45 °C for 10 minutes and then to 9.3 after cooling to 20 °C in a water bath (see Fig. 6.12).



(a)

FIGURE 6.12: (a) The change in temperature and pH over a typical procedure reported elsewhere that makes crystals of hydroxyapatite [40]. (b) The change in temperature and pH over the 17 min. procedure used in this work.

This observation confirmed that it was not necessary to maintain the pH after mixing to complete the reaction. The drop in pH observed occurs as the reaction proceeds and the gel acidifies. The later rise in the pH of the solution can be attributed to the cooling of the solution gel.

A pH of 10.4 was found to be optimal. A pH lower than 10 increased the time needed to crystalize the precipitate. A pH higher than 10.5, required a large volume of 30% (16 M) hydroxide ammonia to be added, larger than that required to dilute the 1 M diammonium hydrogen phosphate stock solution to 0.5 M.

The pH of the solution gel was measured during the reaction. As indicated by the drop in pH, most of the nucleation of the crystals occurred within two minutes of heating. Sufficient time, at least 8 minutes, was needed after that to grow the crystals.

6.9 Using a microwave to make rhombus-shaped particles

Rod, whisker and sphere apatite particles are typically reported in the literature [7]. A test was run to confirm if a rhombus shaped particles could be made. It followed a method in the literature [29], but used a microwave instead of a hot plate.

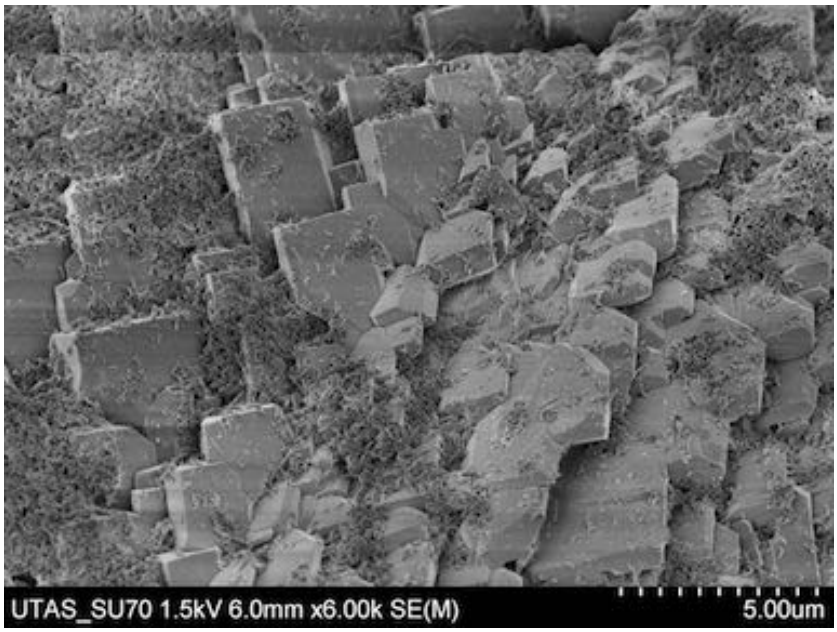
Phosphoric acid with calcium nitrate solution was preheated and mixed while still hot in a commercial microwave and formed rhombus shaped particles, and the results are shown in Fig. 6.13(a) and in Fig. 6.13(b). The yield was low, however and it was difficult to produce these particles in large amounts and so this line of inquiry was not continued. The yield data is reported in Section 6.7.

6.10 Shortening the turnaround time of synthesis by using a microwave

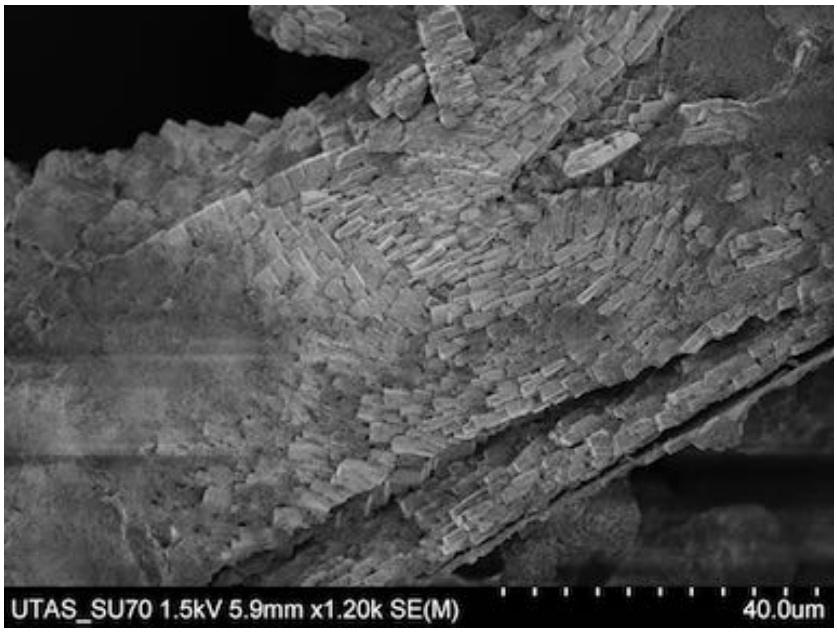
In this Chapter, making apatite with a simple method that used a kitchen microwave was shown (see Fig. 6.1). The microwave did not require modification and heating was done under atmospheric conditions and at full power with the reaction vessel open [18]. No attempt was made to maintain the gel temperature, instead it was left to cool on a bench. This was simple to do and large volumes of gel could be processed rapidly.

As is shown in Section 6.1, it took about 15 minutes to heat and cool the gel. The same type of crystals could be made using the precipitate method, but this method took 4 hours to complete. The shortening of the method would make it possible to repeat it many times within a single day.

The microwave method (described in Section 6.2) produced particles that were



(a)



(b)

FIGURE 6.13: Apatite crystal were made that had a rhombus shape. This can be seen in (Fig. 6.13(a)) and in (Fig. 6.13(b)).

not as complex or as regular as those made with the hydrothermal method and these appeared to be formed from smaller aligned and aggregated particles, as can be seen in Section 6.6. This was seen in reports elsewhere [35], but not commented upon.

Like the hydrothermal method, the gel temperature in the microwave method, reported in Section 6.7 was ramped up, but unlike this method, after microwaving the gel temperature was then slowly cooled. This treatment was reported elsewhere [21], but the focus was not on particle morphology.

The control over the heating conditions of the microwaved gel was limited. Unlike a commercial system, the power of the microwave was not cycled and so it could heat to a fixed temperature (without doing it manually). But it was found that heating the gel at full power with the length of time of heating as the only control over the temperature, together with the size of the volume that was being heated, still provided enough control over the gel's reaction conditions to form the necessary precipitate [18].

7

Conclusion

As reported in this work, a method of practical usefulness was developed that had several advantages over existing methods reported elsewhere. Whereas these other methods took several hours to produce particles, or were performed at a high temperatures and under pressure, the final methods described here, including the microwave method, could be performed with simple apparatus on bench with minimal heating quickly.

The focus of this work was based upon the assumption that a practical way to make implants that are both biocompatible and strong [23, 26] was to assemble small particles of fluorapatite and hydroxyapatite [1, 6]. To make amounts necessary for tissue engineering, particles would have to be made rapidly and in gram quantities per reaction and their shape should be controllable so that they could be aggregated and assembled to form the implant [24].

This work, thus aimed to make sub-micron anisotropic apatite particles simply and rapidly with a yield that was high. It achieved these aims and unexpectedly also developed and expanded some aspects of the methods for making apatite particles, and in some instances produced shapes not seen before, and these are shown in Section 4.5 and in Section 4.6. Some conclusions to these findings are listed next.

A summary of the effects of various factors on the prepared apatite in size and shape follows. As the temperature of the reaction or solution gel rises, the particles were observed to become more crystalline, and whisker in shape. As the concentration of this gel rises, the particles become less crystalline and smaller in size. As its pH rises the particles were observed to become more whisker in shape and larger in size. The pH and temperature were observed to be interrelated, a rise in temperature has a similar effect as an increase in pH and vice-versa. The solvent was found to have a major effect on the particles. Few reports have investigated the relationship of solvents other than water and the formation of a precipitate. In this work, most of the solvents tested were found to stop or reduce the formation of a precipitate. In the few instances where a precipitate was formed, the particles were less regular than if they were formed in water and the shapes produced were unusual and typically prismatic in form. The effect of the type of heating was tested. It was found that for the most part, the particles made did not vary with how they were heated if enough time was given for their formation. However, their crystallinity varied as the final temperature reached varied, with a higher temperature increasing crystallization. The time taken for their formation was either short (seconds to minutes) for microwaving, or long (hours to days) for conventional or hydrothermal heating.

1. **The development of a simple approach in the fabrication of regular anisotropic crystals of apatite**

   Some of the reaction conditions implied in the literature [7] were found to be not necessary. As reported in Section 3.9, the control of the gel pH in an ongoing reaction was not necessary and adding a chelate such as citric acid to the gel was

also not needed when making regular particles. Without a chelate added, the yield from the gel, as described in Section 5.4, was found to be a higher. The yield data is reported in Section 6.7.

Although an incremental improvement, this work demonstrated in Section 6.4 that the gel could be mixed by shaking for a few seconds rather than, as suggested by the literature, stirred for many hours, and this change did not diminish the particles produced.

Furthermore, the hydrothermal reaction, described in Section 5.1, was repeated at a temperature below boiling point, which made the method simpler to run. This work did report in Section 6.3 that the best order for making apatite was the same as that commonly described elsewhere [41]; adding, mixing, heating and then incubating before washing.

An important outcome of this work was that it found that many aspects of the method used in the literature could be either simplified or in some cases completely removed and still produce a precipitate that was acceptable. For instance, precursor chemicals to the reaction, as shown in Section 5.3, did not have to be preheated and could be added by pouring, as shown in Section 5.4.

Some mixing was needed, but, as mentioned in Section 6.7, stirring for extended lengths was found to be unnecessary. Regular particles could be produced by all the methods reported possibly because these were variations on the same general approach as was described in Section 6.2. It was reported in Section 3.2.5 and in Section 3.2.5, however that the optimal approach found was not, in some instances, transferable to the others.

It was also found that a complex solution gel or a complex treatment was more likely to lead to less regular crystals then a simple gel or treatment. It appears that control over shape was lost as the number of the reagents increased. Other than water, for example most solvents reported in Section 3.5 disrupted regular crystal formation.

It was described in Section 3.7 that metal doping of gels created new apatite shapes, but these were all less regular. Adding carbonate, as seen in Section 4.3, also reduced the regularity of particles made. Seeding the gel, as seen in Section 3.9, with small particles was found as well to not improve the regularity of particles.

2. **Making large amounts of regular particles of apatite**

It was found that when a chelate, such as citric, was added to the gel and heated slowly, the particles, illustrated in Section 5.2, were more likely to be regular and as well were observed to contain an internal microstructure. A dipole moment might explain how some of these shapes were produced, and this was shown in Section 3.2. If EDTA was added without citric as described in Section 3.3, the particles produced were irregular, EDTA added with citric, and reported in Section 3.4, produced particles, however that were regular.

It was generally found that when a chelate was added to a gel, such as show in Section 5.4, particle regularity increased, but the yield of the gel was substantially reduced. Slightly less regular particles could be made, as see in Section 5.3, if a chelate was not added and this produced a precipitate to a high yield. It was also found that other additives could be omitted from the gel; as shown in Section 3.8, it was found that it was possible to make regular particles of hydroxyapatite without fluoride present. The yield data is reported in Chapter 6.

Some regularity of particles was lost when chelate or fluoride was not omitted from the gel, but it was believed that this could be somewhat regained with the addition of surfactants (Section 3.6), amino acids (Section 4.1) and some buffers (Section 4.2). In Section 3.11, heating the gel to a higher temperature was reported to increase crystallinity. However, if the temperature became too high and particle morphology was disrupted by bumping in the gel.

It was found that incubation of the gel (which is also called ageing) for at least some time after heating to a low or high temperature was necessary to form a

crystalline precipitate, but this incubation did not have to be at a fixed temperature and it did not have to be run for a long time. Surprisingly, as shown in Section 6.3, slow cooling after rapid heating of the gel was enough to produce regular particles. This observation was unique to this work.

It was shown in Section 6.7 that pH and temperature of the gel were interrelated and their joint adjustment could be used to manage particle morphology. For instance, as seen in Section 4.4, there were certain ranges of pH that were best for particular gel formulations and target temperatures, which produced the most regular particles.

3. **The development of a method that had a short turnaround time**

Depending upon the volume treated with a microwave oven that was used to heat the gel, the method used in this work for making regular particles was shortened, as reported in Section 6.1, from four hours to less than 1 hour. With further optimisation, this was shortened to 15 minutes for processing an 8 mL volume of gel (Section 6.4).

A temperature of over 65 °C and at least 10 minutes of post incubation by cooling was found to be the minimum necessary for creating crystals (Section 6.7). Furthermore, it was found that if the gel was heated to 85 °C, crystal formation became optimal. It was also observed that these particles, when viewed at a high magnification (Section 6.6), appeared to be made from smaller particles.

Unexpectedly, crystals were made that had shapes not seen elsewhere in the literature which could not be explained by existing theories on apatite formation. It was shown in Section 4.5, for instance, that oxalic acid made brick-shaped particles when it was added, dioxane made gull-wing shaped particles when it was used as a partial solvent (Section 4.6) and rhombus-shaped particles were produced when the composition of the gel and how it was heated was changed (Section 6.9).

Various shaped and sized apatite particles were produced by the Author and

described in this work. Those particles that were considered by the Author to be of most of interest to the intended tissue application were those that displayed a uniform morphology. It was considered that particles that were irregular could not be packed in a regular manner in this type of material. Irregular particles were shown to be mono-phasic, i.e. they would have irregular properties, especially those related to their mechanical strength. Particles that were anisotropic rather than isotropic were also of more interest to the Author. These particles are more likely to self-align if injected into the body. They were also more likely to pack uniformly and to display anisotropic properties, which is a prevalent approach taken by the body itself. Also those particles that are smaller (less than 1 micron in size) rather than larger (greater than 1 micron) was also considered to be more useful. Mechanical properties and their deliver into the body tend to improve if the particles used were small. However, there was a trade-off; if the particles were to small (less than 100 nm), they tended to be less crystalline and more amorphous and thus potentially have less mechanically and chemical integrity.

From the initial findings described in this work, and from the difficulties seen in the literature [24, 25], it appears that the long-range alignment of the particles will not be straightforward to achieve. However, in this work short (Section 4.7) and long (Section 4.8) range alignment of particles was observed, but little further control could be exerted over this alignment.

7.1 Future directions in the investigation of ways to make apatite

This work has shown that it was possible to make regular particles of apatites with a method that had (1) a minimum of complexity, (2) a high yield and (3) a short turnaround time. Unexpectedly, it also produced new ways for making particles not seen in the literature and also developed some further insights into how particles might be aligned.

The particles reported here were not aligned and assembled into centimetre-scale monoliths. Learning from the attempts reported in this work, it was considered a non-trivial task, that has so far seen little progress elsewhere [9, 24, 25]. It would require a much more thorough understanding, than what is currently known, about how particles can be induced to align at the centimetre-scale.

A

Appendix

In consultation with the Author's research group, the following draft paper for the journal Chemistry Communications published by the Royal Society of Chemistry was prepared for its submission. The findings reported in this paper were referred to and support some of the findings listed in the main text of the thesis.

Journal Name

COMMUNICATION

Microwave-facilitated synthesis with minimal complexity and turnaround time of highly regular hydroxyapatite crystals

T. S. Lawson,[a] A. C. Grosse,[b] N. L. Kilah,[b] P. N. Nesterenko[b] and B. Paull[b]

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www.rsc.org/

Highly regular and pure crystals of hydroxyapatite were rapidly synthesized. Many of the assumptions implicit in methods used elsewhere were found to be not necessary. The solution gel was shaken and then its temperature ramped up with a microwave and then ramped down by cooling.

An aim of this work is to precipitate large elongated particles of hydroxyapatite, which are themselves micro-constructed from an assembly of smaller particles. Large unidirectional crystals can be more easily aligned mechanically. Crystal that is made from and contains smaller particles has a large surface area, which can be doped with other material. Crystals that are highly regular in shape have more predictable properties and indicate in high crystallinity and purity. Crystals that can be made to different shapes can be tailored to different purposes.

An existing approach, which can take a few days to complete, is simply to add the reactants together and sit them on the bench at room temperature, but this produces spherical particles that are less than 50 nm in size, irregular and devoid of a microstructure. Another approach is to add and mix the reactants together at a temperature above the boiling point of water in a pressure vessel with additives, but this requires specialized equipment and the yields are low, less than 5%.

Generally the more complex and long the method, the more complex and bigger the crystals and the more likely they are to contain a microstructure. What is lacking is a short turnaround method that can be implemented with minimal resources and that can produce this form of crystals.

Many of methods elsewhere are optimal for their intended to use, but what has not been done before is a method that makes highly regular unidirectional crystals, which includes an internal microstructure with a focus on the brevity and minimal complexity of their fabrication.

We hypothesize, however that previous work done on the synthesis of hydroxyapatite is not at its minimal complexity or turnaround time. Repeating, combining and simplifying previous methods will test this hypothesis (see Figure 1). We will also attempt to minimize or remove any treatments, which have no effect on the outcome. In some steps we will test unique and better ways of manufacturing hydroxyapatite.

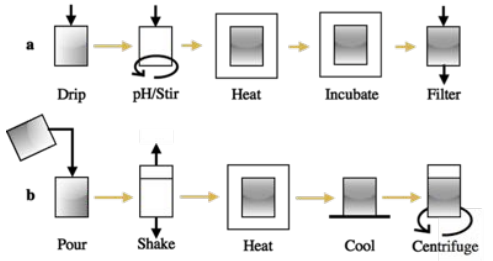

Figure 1 (a) A schema of the procedure for making crystals of hydroxyapatite typically reported elsewhere. (b) The 17 minute assay described in this work.

This work will use the simplest method of making hydroxyapatite, the precipitation method, and to improve upon this method so that it produces the desired outcome. Additional complexities such as the inclusion of additives such as citric acid or EDTA to template the crystals will be avoided. We hypothesize that minimizing the number of reagents in the solution gel will provide greater control over the parameters of the reaction and improve the yield.

The work will determine if the method can be shortened, while still producing a precipitate, which is crystalline. We hypothesize that heating with a microwave to as high a temperature as possible without boiling will substantially shorten the method. Heating with a microwave is known to produce hydroxyapatite quicker than heating conventionally or not heating at all and this will be used.

[a] *Centre for Nanoscale BioPhotonics (CNBP), Macquarie University Sydney, NSW, 2109, Australia. E-mail: tomxlawson@gmail.com, Tel: +61 3 6226 2999*
[b] *Australian Centre for Research on Separation Science (ACROSS), University of Tasmania, Hobart TAS 7001, Australia. Email: brett.paull@utas.edu.au, Tel: +61 3 6226 2121*

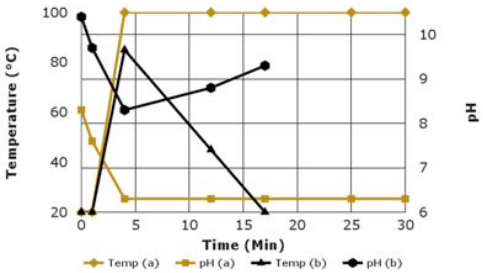

Figure 2 (a) The change in temperature and pH over a typical procedure reported elsewhere that makes crystals of hydroxyapatite. (b) The change in temperature and pH over the 17 min. procedure used in this work.

The work will determine if large complex crystals can be produced, as these crystals are more likely to contain microstructures and be tuneable. Treatments that were reported to increase the size of the crystals will be repeated and those that decrease, avoided. We hypothesis that it is possible to implement more exact controls over conditions of the solution gel and thus greater control over the outcome of its precipitate.

To prepare for the method, diammonium hydrogen phosphate ((NH4)2HPO) at 99% purity and calcium nitrate (Ca(NO3)2.4H2O) at 98% purity (Ajax Chemicals) were as received prepared as 1 M stock solutions and then diluted to 0.5 M and their pH adjusted to 10.4 using 16 M of hydroxide ammonia and 2 M of nitric acid solutions.

3 ml of the diammonium hydrogen phosphate solution diluted with Milli-Q (Millipore) water was poured into a 25 ml glass bottle containing 5 ml of the calcium nitrate solution so at a the 1:1.67 stoichiometric ratio for hydroxyapatite. The bottle was sealed and shaken mechanically (Retsch, MM200), typically for 10 seconds at 10 times a second, and then placed at the centre of a turntable (5 rpm) in a microwave oven with the bottle open. This step was also done by hand and produced the same precipitate. The household microwave (Homemaker, EM820CTM) was run at full power until the gel reached 85°C after 14 seconds.

The bottle was left on the bench for 10 minutes to cool to 45°C. The reaction was stopped, by placing it in water bath until it reached 20°C after 5 min. The precipitate that was formed was washed three times by centrifuging (5810, Eppendorf) at 1000 RPM for 1 min. The washed precipitate was stored in water so that it would not dry out. Each experiment was repeated three times and produced a yield of 0.21g or 84%.

The same synthesis was repeated with 30 ml of diammonium hydrogen phosphate and 50 ml of the calcium nitrate reacted in a 100 ml plastic container and produced a yield of 86%. The gel reached 85°C after 70 seconds of microwaving, cooled to 45°C after 30 minutes on the bench and was washed at 2000 RPM for two minutes each three times. To calculate the yield, the precipitate was dried in a vacuum (~ 50 mm Hg), washed with water and then 95% ethanol, dried again by suction and then dried at 60°C to 2 hours under the same vacuum.

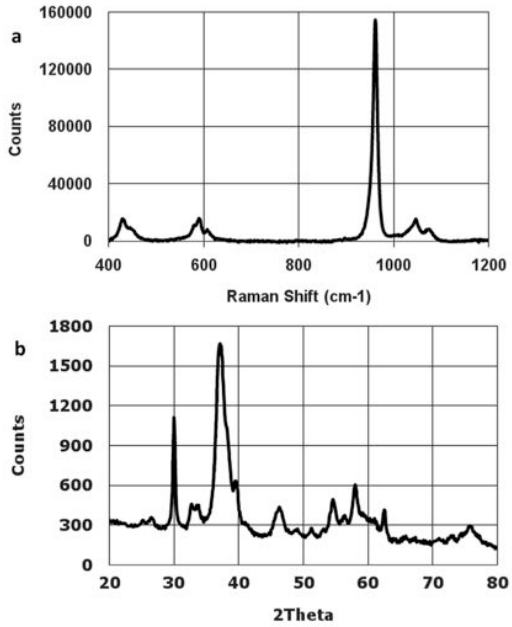

Figure 3: (a) A Raman spectra and a (b) XRD spectra of the precipitate first shaken at 10 times a second for 10 seconds.

Energy-dispersive X-ray spectroscopy (EDX) was performed with a Hitachi SU-70 field emission scanning electron microscope (SEM) at 7 kV on dried precipitant and the calcium to phosphate weight ratio was measured at 2.1:1, close to the theoretical value of 2.15. For X-ray diffraction (XRD) the dried precipitate was scanned with a D2 PHASER (Bruker), and for Raman, scanned with a Renishaw inVia microscope using a 785 nm laser (see Figure 3). These measurements confirmed the precipitate was hydroxyapatite.

The precipitate was heated at 10°C a minute to 1200°C in nitrogen using a Setaram LABSYS evo system. Thermogravimetric analysis (TGA) showed no loss of weight above 500°C, which suggests no carbonated hydroxyapatite. For imaging, the SEM was run at a 2.5 to 40 K magnification and 1.5 kV acceleration. For the zeta potential (calculated at -18.3 mV) and size, a Zetasizer (Malvern Nano ZS Zetasizer) tested precipitate in neutral water. The wet aggregated particle was 1 µm in length, longer than the approx. 0.5 µm observed in the dry precipitate with the SEM (see Figure 4).

SEM images were analysed with ImageJ 1.48V (NIH) and SPSS 22V (IBM). Images were converted to 8-bit binary processed with Cell Magic Wand (by Theo Walker). A t-test was run and the mean and 95% confidence interval calculated (n = 30) (see Table 1). To compare the effect of shaking, a one-way ANOVA (for length, $F(3, 116) = 39.7$, $p = .000$, for width $F(3, 116) = 12.6$, $p = .000$, and for the aspect ratio, $F(3, 116) = 2.3$, $p = .077$) and a Tukey post-hoc test (for not shaking vs. shaking 445 +/- 34 nm, $p = .000$) were performed. Normality was tested with Kolmogorov-Smirnov and Shapiro-Wilk.

In order to investigate the effect of how the gel was mixed, the solution was either shaken or stirred. Without at least

Table 1: The mean and 95% confidence interval of the length, width and aspect ratio (nm) of the crystals after shaking at different speeds for 10 seconds.

Shaking frequency per second	Mean length of crystal	Mean width of Crystal	Mean length/width ratio
No Shaking	445 +/- 34	165 +/- 22	3 +/- 0.4
10	296 +/- 28	132 +/- 15	2.5 +/- 0.4
20	277 +/- 24	104 +/- 14	3 +/- 0.4
30	252 +/- 26	106 +/- 12	2.5 +/- 0.3

some mixing, the crystals produced cannot be regular, but we found that the gel could be sufficiently, homogenised by shaking and this was much faster. If the gel is shaken instead, mixing can be kept to a minimum and the size of crystals can be kept to a maximum. This finding has not been reported elsewhere.

In this work crystals were produced that were as large as about 0.5 µm in size (see Figure 4), which is about 10 times larger than what was reported elsewhere. Shaking can be short, about 10 seconds at 10 times a second to homogenize the solution. At higher speeds of shaking (20 to 30 times a second), although not statistically different, the particles appeared to become smaller (see Figure 4).

In order to investigate the effects of mixing and heating and cooling on the pH, the pH of a gel was measured. The pH dropped from 10.4 to 9.7 after first mixing and then to 8.8 after heating to 85°C and finally to a low of 8.3 two minutes after heating before rising to a 8.8 after cooling to 45°C for 10 minutes and then to 9.3 after cooling to 20°C in a water bath (see Figure 2).

This observation confirmed that it was not necessary to maintain the pH after mixing to complete the reaction. The drop in pH observed occurs as the reaction proceeds and the gel acidifies. The later rise in the pH of the solution can be attributed to the cooling of the solution gel.

The concentration of the precursor reagents was varied. Crystallisation of the precipitate was found to be dependent on the concentration of the solution gel. If the concentration of the chemicals to be mixed is greater than 0.5 M, two phases are developed in the precipitate, one crystalline and the other amorphous.

Different means of heating were tested. It was found that heating with a microwave was produced a precipitate about 16 times faster than if a hotplate was used and about 48 times faster if the reaction was at room temperature. If turnaround time is important, a microwave should be used.

To investigate the effect of a fixed vs. ramped heating and cooling, the solution gel was either heated rapidly and cooled slowly or placed in an oven at a fixed temperature, incubated for 17 minutes and then cooled rapidly in a bath of water. Contrary to results elsewhere, ramping the temperature up and down was as effective as incubating at a fixed temperature. It was simpler to implement ramping rather then a fixed temperature incubation with a domestic microwave and the crystals were more regular.

The pH of the solution gel was measured during the reaction. As indicated by the drop in pH, most of the nucleation of the crystals occurred within two minutes of

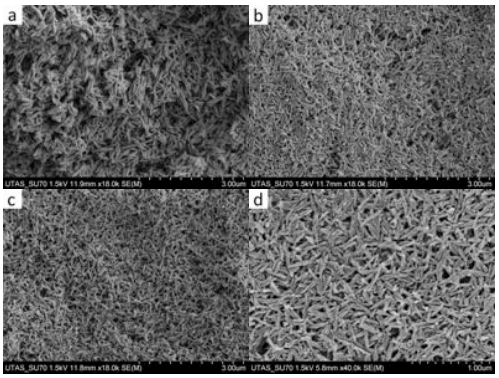

Figure 4: SEM images of the crystals after (a) not shaking, and shaking for (b) 10, (c) 20 and (d) 30 times a second at a higher magnification, and shows the assemblage of smaller particles.

heating. Sufficient time, at least 8 minutes, was needed after that to grow the crystals.

A pH of 10.4 was found to be optimal. A pH lower than 10 increased the time needed to crystalize the precipitate. A pH higher than 10.5, required a large volume of 30% (16 M) hydroxide ammonia to be added, larger than that required to dilute the 1 M diammonium hydrogen phosphate stock solution to 0.5 M.

The method was tested at 8 and with 80 ml volumes of solution gel. The reaction was completed in 17 minute with an 8 ml volume and, for the 80 ml volume, in 40 minutes. The same crystals were produce, which suggests the method is scalable. The turnaround time for the 80 ml was shorter reports elsewhere that processed the same volume.

In order to do investigate the effect of which reagent to add to which, the order of adding reagents was varied as well as the speed of their addition. Pouring the phosphate into the calcium produced the most regular and crystalline particles in the shortest possible time. It was also found that it was not necessary to preheat the reagents.

The final temperature to solution gel was heated too was varied. Heating the gel rapidly to 85°C produced particles that were more crystalline and regular than heating either slowly or rapidly to a temperature above or below that. Temperatures below 65°C were insufficient for crystal formation, and temperatures above 85°C were over sufficient; crystal formation was disrupted by bumping.

In summary, this work simplifies and optimizes the core aspects of hydroxyapatite synthesis so that it can be completed rapidly and produces a precipitate of crystals with some degree of control. It found that it is not necessary to stir. It is not necessary to incubate at a fixed temperature or for a long period of time. It is not required to maintain the PH throughout the reaction. Additives are not necessary, nor is it necessary to use a pressure vessel.

It observed and recognized the interesting phenomena of the drop and then rises in the pH of the solution gel. Finally it observed an assemblage of smaller 50 nm long particles that formed each crystal, something that has shown before, but not specifically commented upon. The importance of the period of time, between just after pouring the two solutions together

and two minutes after heating when the pH stabilizes at 8.3, warrant further attention.

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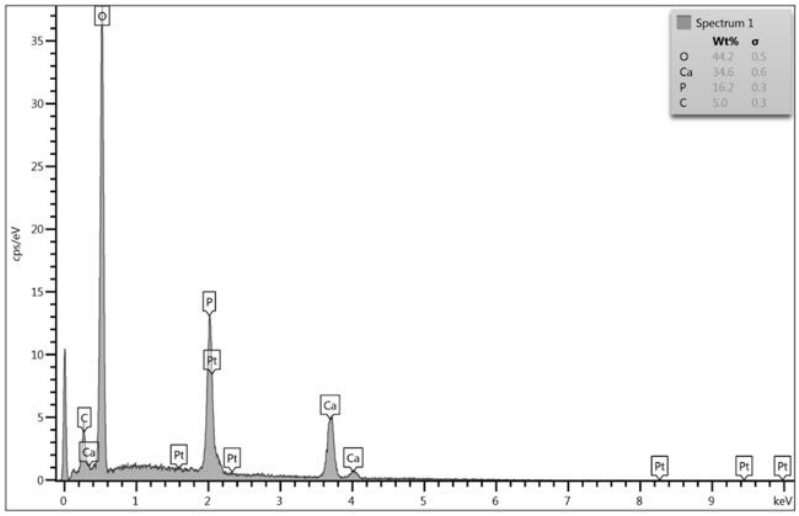
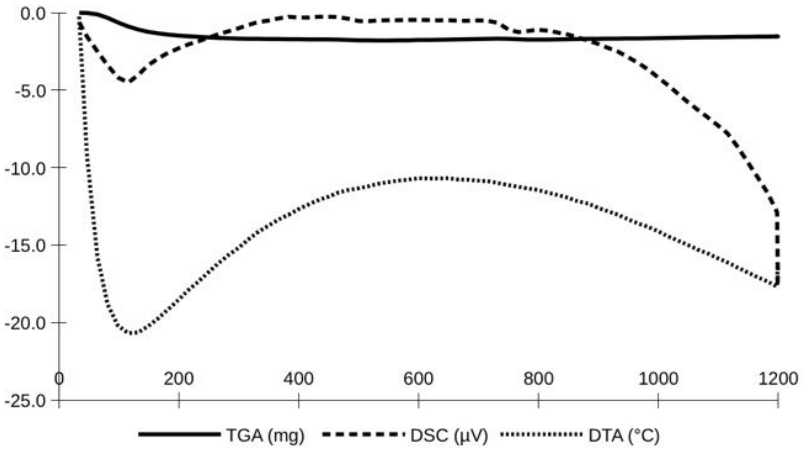


(a)



(b)

FIGURE A.1: EDX and TGA analysis of precipitate indicated hydroxyapatite. The results from the EDX matched the correct ratio of calcium to phosphate ions for hydroxyapatite (Fig. A.1(a)). TGA results indicated that no materials apart from water were present (Fig. A.1(b)).

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